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(NASA-CR-160567) THE EFFECT OF VARIETY AND
MATURITY ON THE QUALITY OF FREEZE-DRIED
CARROTS. THE EFFECT OF MICROWAVE BLANCHING
ON THE NUTRITIONAL AND TEXTURAL QUALITY OF
FREEZE-DRIED SPINACH Final Report (Texas

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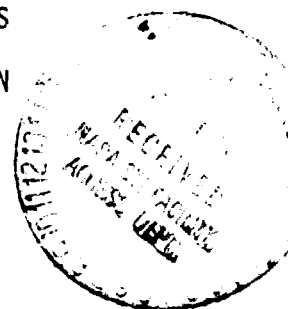
THE EFFECT OF VARIETY AND MATURITY ON
THE QUALITY OF FREEZE-DRIED CARROTS

THE EFFECT OF MICROWAVE BLANCHING ON THE NUTRITIONAL
AND TEXTURAL QUALITY OF FREEZE-DRIED SPINACH

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Final Report

Prepared by
ADRIANCE LABORATORY
DEPARTMENT OF HORTICULTURAL SCIENCES
TEXAS AGRICULTURAL EXPERIMENT STATION
TEXAS A&M UNIVERSITY



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FINAL REPORT

THE EFFECT OF VARIETY AND MATURITY ON
THE QUALITY OF FREEZE-DRIED CARROTS

THE EFFECT OF MICROWAVE BLANCHING ON THE NUTRITIONAL
AND TEXTURAL QUALITY OF FREEZE-DRIED SPINACH

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PART I

THE EFFECT OF VARIETY AND MATURITY
ON THE QUALITY OF
FREEZE-DRIED CARROTS

LITERATURE REVIEW

Background

The carrot, Daucus carota, gets its name from the French word carotte, which in turn comes from the Latin carota. It has been known since ancient times and is believed to have originated in Afghanistan and adjacent areas (Boxwell, 1949).

The carrot is a biennial of the Umbelliferae or parsley family. The genus Daucus, to which the carrot belongs, contains about 60 species, some of which are native to North America. Very few of them are cultivated (Thompson, 1939).

Our common carrot is called the Mediterranean type, because it has long been known in Mediterranean countries and was probably developed there from types carried from Asia Minor. All varieties of importance in this country are deep orange in color; although yellow and even white types are known (Boswell, 1949).

Carrots are an excellent source of vitamin A and a good source of thiamine and riboflavin. The carrot is also high in sugar.

Freeze Drying

Freeze drying is the more prominent example of evaporation by sublimation. In freeze drying, water is removed as a vapor from a frozen substance (King, 1971). The process is carried out under a vacuum to provide a high vapor diffusion potential and is accelerated by supplying heat (Holdsworth, 1971).

As a method of food preservation, freeze drying, as a rule, produces the highest quality product obtainable by any drying method.

King (1971) lists the following reasons for the high quality of freeze-dried products:

1. The structural rigidity of the frozen material prevents collapse of the solid matrix and results in a porous, non-shrunken structure which facilitates rapid rehydration.
2. The low processing temperature and the absence of liquid water reduces degradative reactions such as nonenzymatic browning, protein denaturation, and enzymatic reactions.
3. The absence of a liquid state prevents the movement of soluble matter from one region of material to another.
4. The low temperature reduces the loss of flavor and aroma.

The high cost of freeze drying, compared to other means of food preservation, has hindered progress. Freeze drying has found increasing application for foods which can carry the added processing costs, and for foods where convenience and weight reduction are the overriding concern such as military rations, space foods, and foods for campers (Longan, 1973).

Compression - Rehydration

Freeze drying results in a major weight reduction with little or no reduction in volume. Compression can give a high degree of volume reduction; allowing for more compact packaging and shipping.

If they are rehumidified to provide plasticity before compression, freeze-dried foods have been found to provide a compressed food with good rehydration and textural properties (Brockman, 1970; Mackenzie and Luyet, 1969).

Lampi (1967) described three techniques used to effectively plasticize freeze-dried foods to moisture levels of 5 to 20%:

1. Spraying the freeze-dried product until the desired level of moisture is reached.
2. Stopping the freeze drying operation when the product reaches the desired moisture level.
3. Allowing freeze-dried foods to equilibrate in a humidified chamber until the material absorbs the desired amount of moisture.

Curry (1974) found a significant increase in rehydration using the equilibrium method of plasticization over the spraying method. This was probably due, in the latter method, to over-compression of the outer cells which resulted in the excessive rehydration times.

Rushing (1975) determined that the interaction of moisture and temperature had a profound effect upon the rehydration characteristics of carrot bars. The optimum precompression variables were found to be 7% moisture and 32.2°C. In most cases the shedding and rehydration times are directly proportional to the pressure and dwell times used in compression (Macpherson, 1973; Rushing, 1975). While compression of freeze-dried carrots does not cause detectable chemical changes, it presents problems in fragmentation. The degree of fragmentation is also proportional to the pressure and dwell time utilized during the compression phase (Lampi, 1967; Macpherson, 1973; Rushing, 1975).

Bennet (1976) found that both rehydration rate and rehydration ratio were inversely related to maturity in carrots, with carrots harvested at 103 days having the most desirable rehydration ratio.

Chemical

While a maximum sugar content would seem desirable in carrots

for fresh market, high levels of sucrose can have a detrimental effect upon dried products. Curry (1974) showed that incorporation of sucrose into carrot slices during blanching can have an adverse effect on rehydration rates of freeze-dried compressed carrots. Carlton and Peterson (1963) reported positive correlations between soluble solids and dry matter, total sugars and non-reducing sugars. Bennet (1976) found that total sugar was inversely related to rehydration rate; while cellulose was positively correlated to rehydration rate at certain stages of maturity. Sistrunk et al. (1967) determined that total sugars increased for each successive harvest although not always significantly.

Morphological

The basic morphological unit of the plant, the cells, are associated with each other in various ways forming coherent masses of tissues. These cells are of various types, thus allowing many different combinations within the plant. The vascular system of plants is composed of xylem, the principal water conducting tissue; and phloem, the food conducting tissue (Esau, 1967).

After germination, almost as soon as the first leaf is expanding, a secondary thickening starts in the hypocotyl and root. The stele of the root is diarch, and a cambium is laid between the primary phloem and the primary xylem. The two isolated fragments of cambium are joined together as a result of the development of more cambium in the region of the protoxylem. This cambium soon becomes circular in outline in transverse view, and produces secondary xylem towards the center and secondary

phloem, and in the mature root these zones are easily recognized because of the lighter color of the secondary xylem (Berrie, 1977).

Pepkowitz et al. (1944) found an inverse relationship between size-types of carrots and carotene content per unit volume. This indicated that the large type strains had less carotene than the small types. However, no association was found between the shape ratio (width x length) and carotene concentration in the carrot cultivars considered.

Wisakowsky (1977) determined that the tissue ratio (core/cortex) of carrots had no effect on the rehydration ratio or rheological parameters in stress-relaxation for freeze-dried compressed carrot bars.

Rheology

Rheology has been defined as a science devoted to the study of deformation and flow. Examples of rheological properties are time dependent stress and strain behavior, creep, stress-relaxation, and viscosity.

Viscosity is resistance to flow indicated by the coefficient of viscosity which is the ratio of shearing stress to shearing rate and given in poises. The units for poise are $\frac{\text{dynes} \cdot \text{sec.}}{\text{cm}^2} = \text{poise}$ or $\frac{\text{lb.} \cdot \text{sec.}}{\text{ft}^2}$ (Mohsenin, 1970).

A Newtonian liquid is an ideal fluid in which the relationship between shear stress and shear rate is a straight line passing through the origin. This relationship is shown in Figure 1.

A relationship where shear stress and shear rate are non-linear indicates a non-Newtonian liquid. If the flow curve is convex to the shear stress axis, the flow is called pseudoplastic. If the flow is concave to the shear stress axis, the flow is called dilatant. An

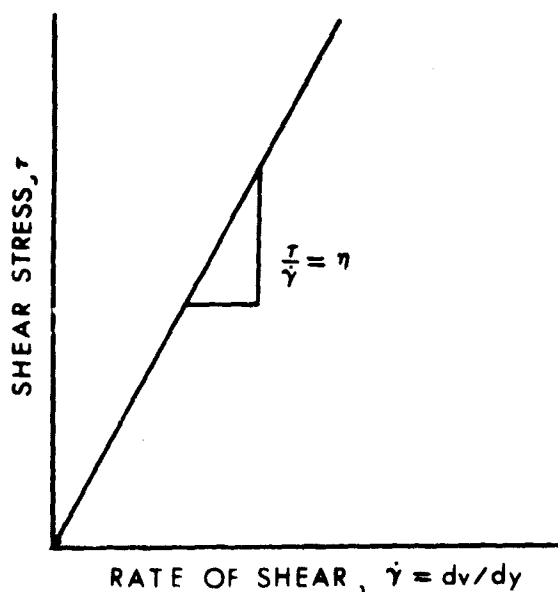


Figure 1. Relationship of shear rate to shear stress in a Newtonian liquid (Mohsenin, 1970).

illustration of non-Newtonian flow is presented in Figure 2. Apparent viscosity as applied to non-Newtonian liquids is the viscosity of a Newtonian liquid exhibiting the same resistance to flow at the chosen shearing stress or shear rate. It is determined from the slope of a straight line connecting the chosen point on the non-linear curve to the origin (Mohsenin, 1970).

Apparent viscosity should not be confused with effective viscosity which is the viscosity the fluid would have if it were Newtonian (Darby, 1976). Harper et al. (1978) have described a back extrusion apparatus and shown it to be a reliable means of measuring a viscosity index in plasma protein gels. They defined the index as:

$$I = \frac{F_{\max}}{B_{\max}}$$

where F_{\max} is the maximum force (dynes) required to move a plunger;

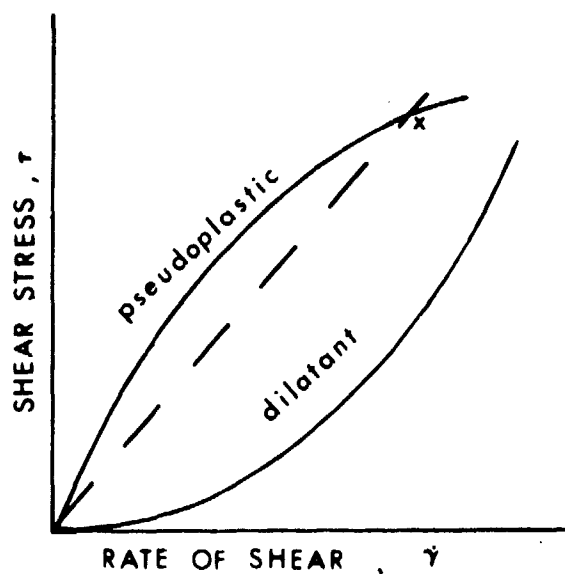


Figure 2. Quazi-viscous or non-Newtonian flow (Mohsenin, 1970).

B_{max} is the minimum value of a geometric factor dependent on plunger, sample, and tube size.

Using these equations Morgan (1978) derived an equation that could be used to give a value for effective viscosity using the back extrusion method.

Viscosity of carrot powder was measured in an effort to determine if this rheological property could be used as an index of quality for the reconstituted freeze-dried and compressed carrots. Although much work has been done on shear and its relationship to the quality of freeze-dried carrots (Longan, 1973; Hruzek, 1973; Bennet, 1976; Wisakowsky, 1977), little has been done on viscosity of carrot purees and its relationship to desirable characteristics. The back extrusion method was chosen due to the small amount of sample needed, ease of measurement, and simplicity of calculation.

Color

The average carotenoid concentrations of carrots reported in the literature vary considerably. Some relationship has been shown between carotene concentration and intensity of pigmentation. Smith and Otis (1941) have shown that light-colored carrots have only one-tenth the carotene content of more highly pigmented varieties. Weckel et al. (1962) correlated carotene concentrations with the Hunter Color Difference Meter values, but found that none of the correlations were high enough to suggest use of the methods for carotene determinations. High correlation has been shown between Hunter color attributes and the logarithm of carotenoid content of raw and canned sweet potato roots (Ahmed and Scott, 1962).

Hruzek (1973) studied 20 carrot varieties to delineate those having desirable quality attributes. He determined that Gardner color values were a desirable means of predicting the processed quality of carrots. Bennet (1976) found that Gardner "a" value and "a/b" ratio was directly related to the rehydration characteristics of individual carrot cultivars.

Summary

Freeze drying of foods offers many advantages over conventional dehydration. Hanson (1961) and Ponting et al. (1973) have shown the importance of optimal varieties for freeze drying and others have shown that maturity can also effect the quality of the finished product.

Since different carrot cultivars vary in composition and adaptability to processing, a need exists to determine the varieties of carrots and the optimum stage of maturity for quality attributes such as appearance, flavor, texture, and nutritive value.

MATERIALS AND METHODS

Raw Product

Carrots of three accepted commercial cultivars, Imperator, Chantenay Red Cored, and Danvers 126, were planted at the Texas A&M University Research Farms on October 1, 1977. Appropriate quantities of each cultivar were harvested at 15 day intervals, beginning at 80 days, for evaluation. These cultivars were selected based upon prior knowledge of existing disparities in their physical and chemical composition.

Processing Procedure

The carrot samples were washed and hand peeled immediately after harvesting. A portion of each cultivar was used for fresh product analysis. The remaining carrots were cubed into 1 cm pieces, steam blanched for four minutes to a negative catalase-peroxide endpoint, quenched in ice water, and frozen at -28.8°C ., subsequent to freeze drying.

Freeze drying was accomplished in a model FFD-40 Sublimator manufactured by the Virtis Company, Gardiner, New York. The samples were freeze-dried for 48 hours at a condenser temperature of -51.1°C and a shelf temperature of 32.2°C . The final moisture content ranged between 2.3 to 2.6%.

At this point, random samples of the carrot pieces were selected to be made into powders with a Wiley Mill utilizing a number 30 screen. These powders were subsequently rehydrated for use in rheological testing.

The freeze-dried carrot samples were plasticized at 12% moisture

and 32.2°C prior to compression in the manner presented by Rushing (1974). The samples were compressed with 250 psi of pressure for 30 seconds in a rectangular stainless steel cell as shown in Figure 3. The finished bars were then refreeze-dried to remove the moisture of plasticization. The carrot bars were then vacuum packed in #303 cans and stored at -51.1°C until subsequent evaluation.

Core/Cortex Ratio

Core/Cortex ratio was determined by measuring the area of a fresh carrot cross section and the area of the core at the same cross section. The area of the core was designated core and the value found by subtraction of the core area from the total area was designated as the cortex. This was done at three locations per carrot: at the crown, at 1/3 the length, and at 2/3 the length. The average value of the three was used for analysis.

Gardner Color

Color was measured with a Gardner Automatic Color Difference Meter, Model XL-10A, calibrated with the Gardner color standard for sweet potatoes. Readings were taken on fresh and freeze-dried samples. Thirty gram samples were pureed in a Waring Blender with an equal weight of water. The puree was then placed in an optically clear cup and allowed to sit for 10 minutes to allow air bubbles to rise to the surface. Gardner color values of "L", "a", and "b" were measured. "L" is considered a measure of lightness of the sample; "+a" a measure of redness; "-a" a measure of greenness; "+b" a measure of yellowness; and "-b" a measure of blueness.

Alcohol Insoluble Solids

Alcohol insoluble solids were determined by the method of the A.O.A.C. (1975). Twenty grams of ground sample were weighed into a 600 ml beaker. The sample was then simmered with 300 ml of 80% ethyl alcohol for 30 minutes, filtered through pre-weighed filter paper, washed with additional alcohol, and dried for two hours at 100°C. The filter paper with the remaining sample was then reweighed and percent alcohol insoluble solids was calculated.

Viscosity

Effective viscosity was determined by the back extrusion method of Harper et al. (1978) with the recommendations of Morgan (1978). This method was a constant strain rate approach. The back extrusion cell, illustrated in Figure 3, was used in determining the maximum shear stress required to move the carrot puree through the annulus between the plunger and the test tube wall.

Two grams of carrot powder were added to a 16X125 mm screw top glass test tube. Eight ml of distilled water were then added and the purees were allowed to rehydrate for one hour prior to testing.

Loading was accomplished on an Instron Model 1122 universal testing machine. Load rate was 200 mm/min., chart speed was 500 mm/min., and full scale was one Newton. The typical penetration-force plot obtained is presented in Figure 4.

The effective viscosity was calculated from the following equation:

$$n_E = 739.476 \times \frac{F_p}{L_p}$$

where: n_E is the effective viscosity in poise

739.476 is a predetermined constant

F_p is the maximum force from the chart in Newtons

L_p is the length the plunger travels in the sample from the chart in cm.

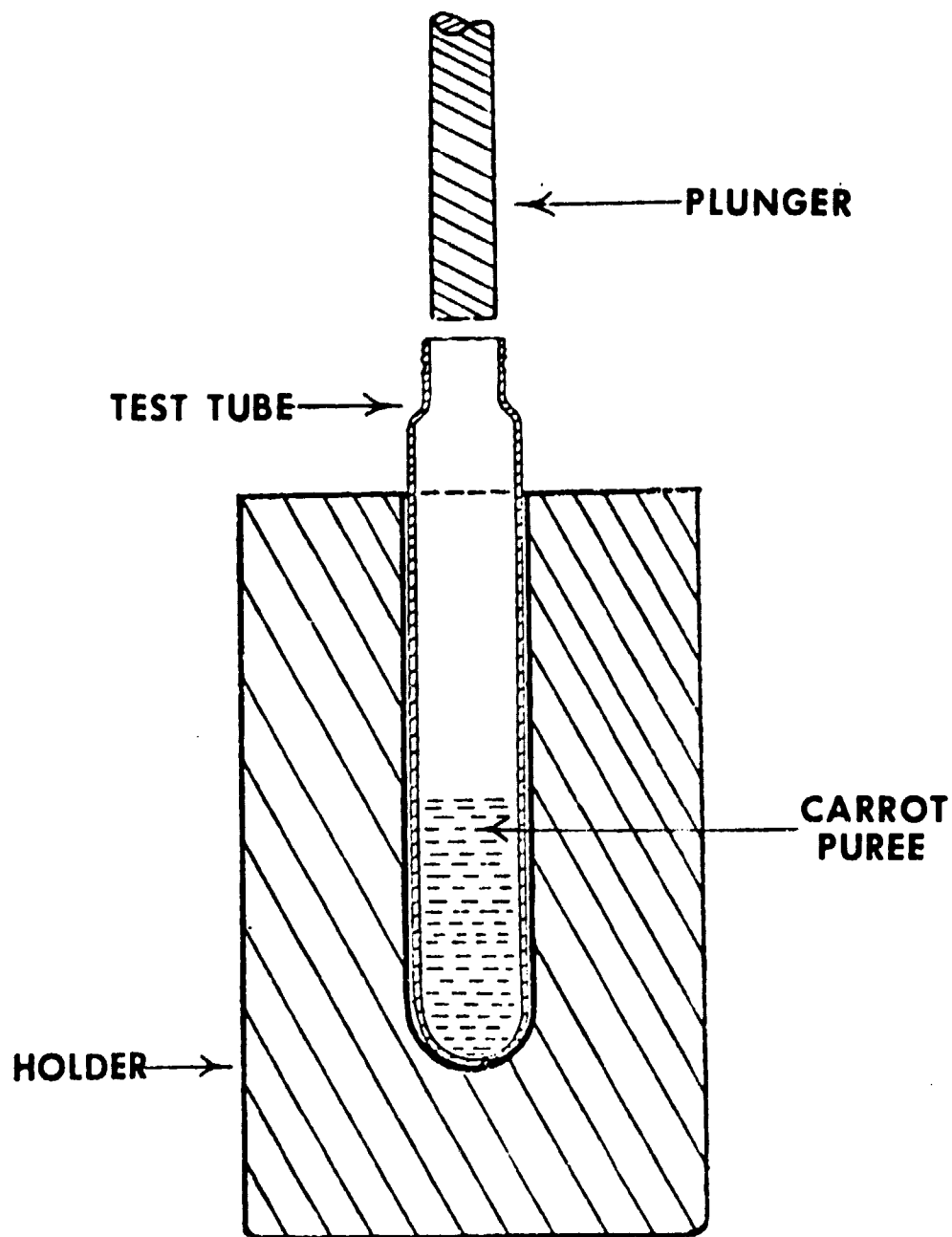


Figure 3. Back extrusion cell used to determine effective viscosity of rehydrated carrot powders.

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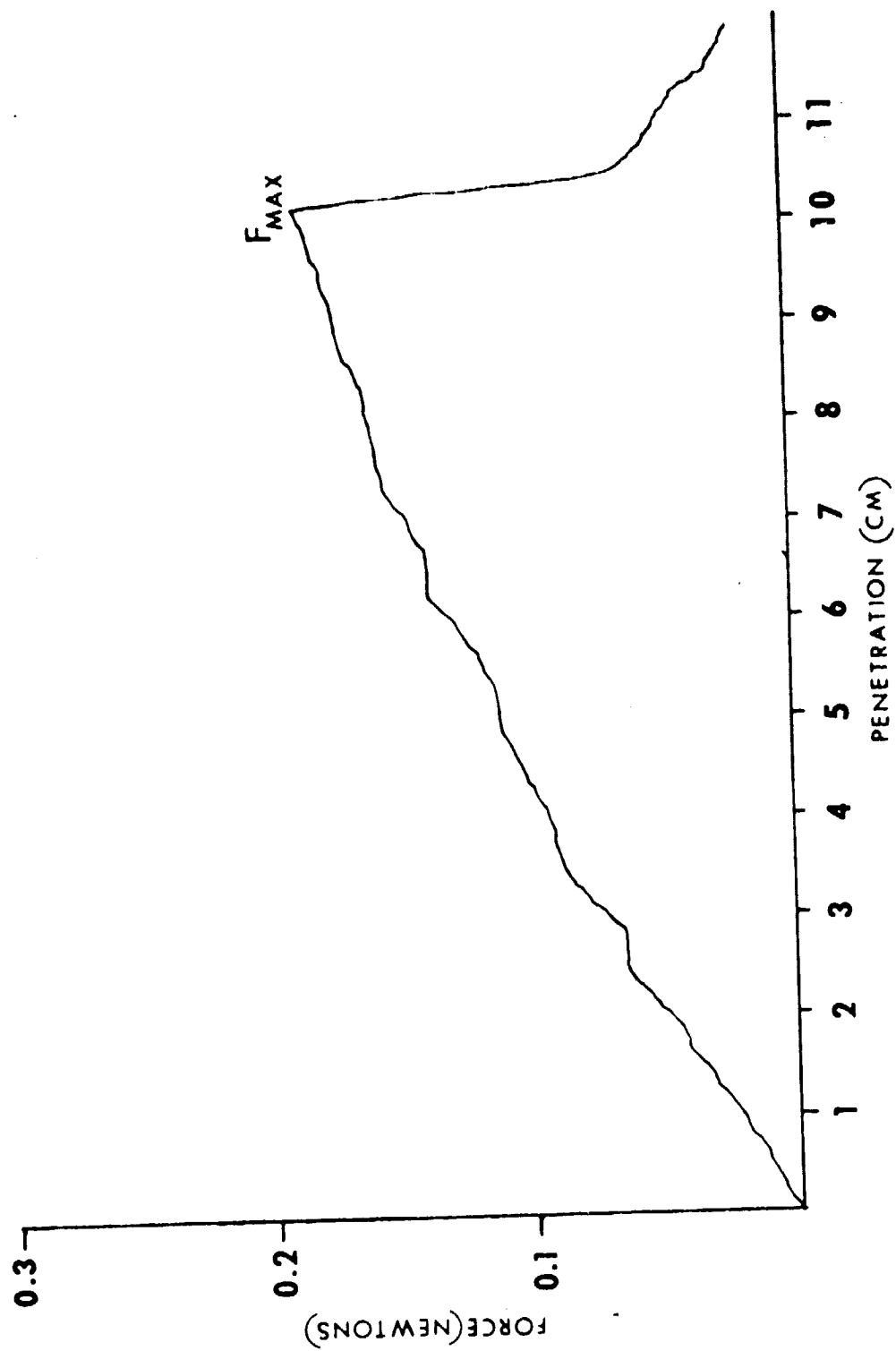


Figure 4. Typical force-penetration plot obtained for carrot purees on Instron loading machine.

RESULTS AND DISCUSSION

Gardner Color

Color intensity and uniformity are important factors in determining the grade of processed carrot products. Environmental conditions and varietal differences have been reported to exert the greatest influence upon carrot color (Bradley and Smittle, 1965). Differences in Gardner color values between carrot cultivars were not as great as those observed between fresh and freeze-dried carrots. The largest differences between fresh and freeze-dried carrots occurred at 80 days after planting.

Gardner "L" Values. The mean Gardner "L" values for fresh and freeze-dried carrots appear in Table 1 and are graphically illustrated in Figure 5. The range for Gardner "L" values for fresh carrots was from 51.0 for Danvers 126 at 80 days to 60.0 for Imperator at 110 days. All cultivars reached a peak for lightness value at 110 days after planting. Danvers 126 showed the least amount of variation throughout the harvests. Values for freeze-dried carrots ranged from 42.4 for Chantenay Red Cored at 80 days to 53.5 for Imperator at 110 days. Loss of lightness was probably due to the dull, water soaked appearance incurred during processing.

The analysis of variance for "L" color is shown in Appendix A. Variety, maturity, and condition, fresh or freeze-dried, were all shown to be significantly related to "L" color ($Pr > F = .0001$). In addition, variety, maturity, and condition were shown to have significant interactions ($Pr > F = .0001$).

Table 1. Effect of harvest date on Gardner color value "L" of fresh and freeze-dried carrots of three cultivars.

Cultivar	Fresh Carrots				Freeze-dried Carrots			
	Days After Planting				Days After Planting			
	80	95	110	125	80	95	110	125
	Mean Gardner "L" Value				Mean Gardner "L" Value			
Danvers 126	51.0 ^{hi}	53.3 ^{de}	53.1 ^{def}	52.3 ^{efg}	43.9 ^m	50.2 ⁱ	51.6 ^{gh}	50.8 ^{hi}
Imperator	52.5 ^{defg}	55.8 ^c	60.0 ^a	55.6 ^c	46.6 ^k	53.2 ^{def}	53.5 ^d	51.3 ^h
Chantenay Red Cored	52.2 ^{fg}	55.5 ^c	57.4 ^b	52.6 ^{def}	42.4 ⁿ	45.5 ^l	47.7 ^j	44.3 ^m
Standard Deviation	0.79	1.37	3.48	1.82	2.13	3.88	1.19	3.90
Overall Mean	51.9	54.9	56.8	53.5	44.3	49.6	52.1	48.8

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Means are of four determinations.

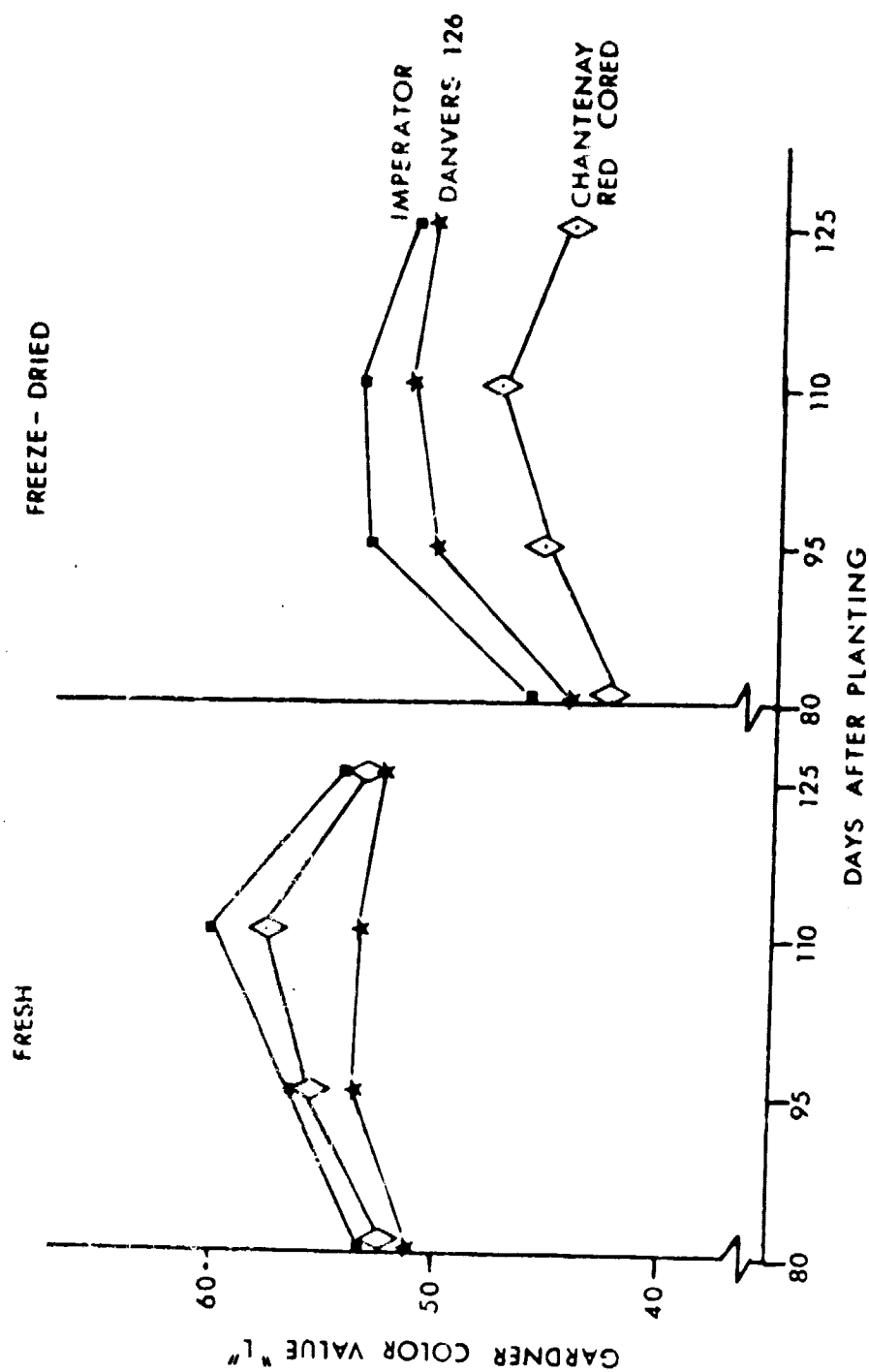


Figure 5. Effect of harvest date on the Gardner Color "L" values of fresh and freeze-dried carrots of three cultivars.

Gardner "a" Values. The mean Gardner Color "a" values for fresh and freeze-dried carrots are presented in Table 2 and graphically illustrated in Figure 6. Gardner "a" values for both fresh and processed carrots increased throughout the growing period with the exception of Chantenay Red Cored which dropped in "a" value between 80 and 95 days for the fresh product. It is postulated that the unexpected drop in "a" value and slow recovery may have been due to the interference of other pigments such as chlorophyll. Gardner "a" values for the fresh product ranged from 21.3 for Chantenay Red Cored at 80 days to 32.5 for Danvers 126 at 125 days. The freeze-dried carrots ranged from 15.6 for Chantenay Red Cored at 80 days to 27.7 for Danvers 126 at 125 days.

Analysis of variance for "a" color is shown in Appendix B. Variety, maturity and condition were all shown to be highly significant related to "a" color ($Pr > F = .0001$). Interactions between variety, maturity, and condition were also shown to be highly significant ($Pr F = .0001$).

Gardner "b" Values. Gardner "b" values for fresh and freeze-dried carrots are shown in Table 3 and graphically illustrated in Figure 7. Both fresh and freeze-dried carrots increased in "b" value from 80 to 95 days and showed a decline to 125 days. Gardner "b" values for fresh carrots ranged from 31.1 for Chantenay Red Cored at 80 days to 35.3 for Imperator at 95 days after planting. Freeze-dried carrots varied from 26.4 for Imperator at 80 days to 33.7 for the same cultivar at 95 days. The Imperator did appear to be a more yellow carrot which could account for the higher "L" and "b" values for this cultivar.

Analysis of variance for "b" color is shown in Appendix C. Variety,

Table 2. Effect of harvest date on Gardner color value "a" of fresh and freeze-dried carrots of three cultivars.

Cultivar	Fresh Carrots			Freeze-dried Carrots		
	Days After Planting			Days After Planting		
	80	95	110	80	95	110
	Mean Gardner "a" Value			Mean Gardner "a" Value		
Denvers 126	27.6 ^d	30.0 ^{bc}	31.1 ^b	18.5 ^h	21.5 ^f	24.2 ^e
Imperator	27.5 ^d	29.5 ^{bc}	30.8 ^b	17.0 ⁱ	20.3 ^g	23.2 ^e
Chantenay Red Cored	26.5 ^d	27.3 ^{fg}	23.4 ^e	15.6 ^j	21.8 ^f	23.6 ^e
Standard Deviation	0.61	4.88	4.36	1.45	0.89	0.50
Overall Mean	27.2	26.9	28.4	17.0	21.3	23.7
			29.8			26.1

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Means are of four determinations.

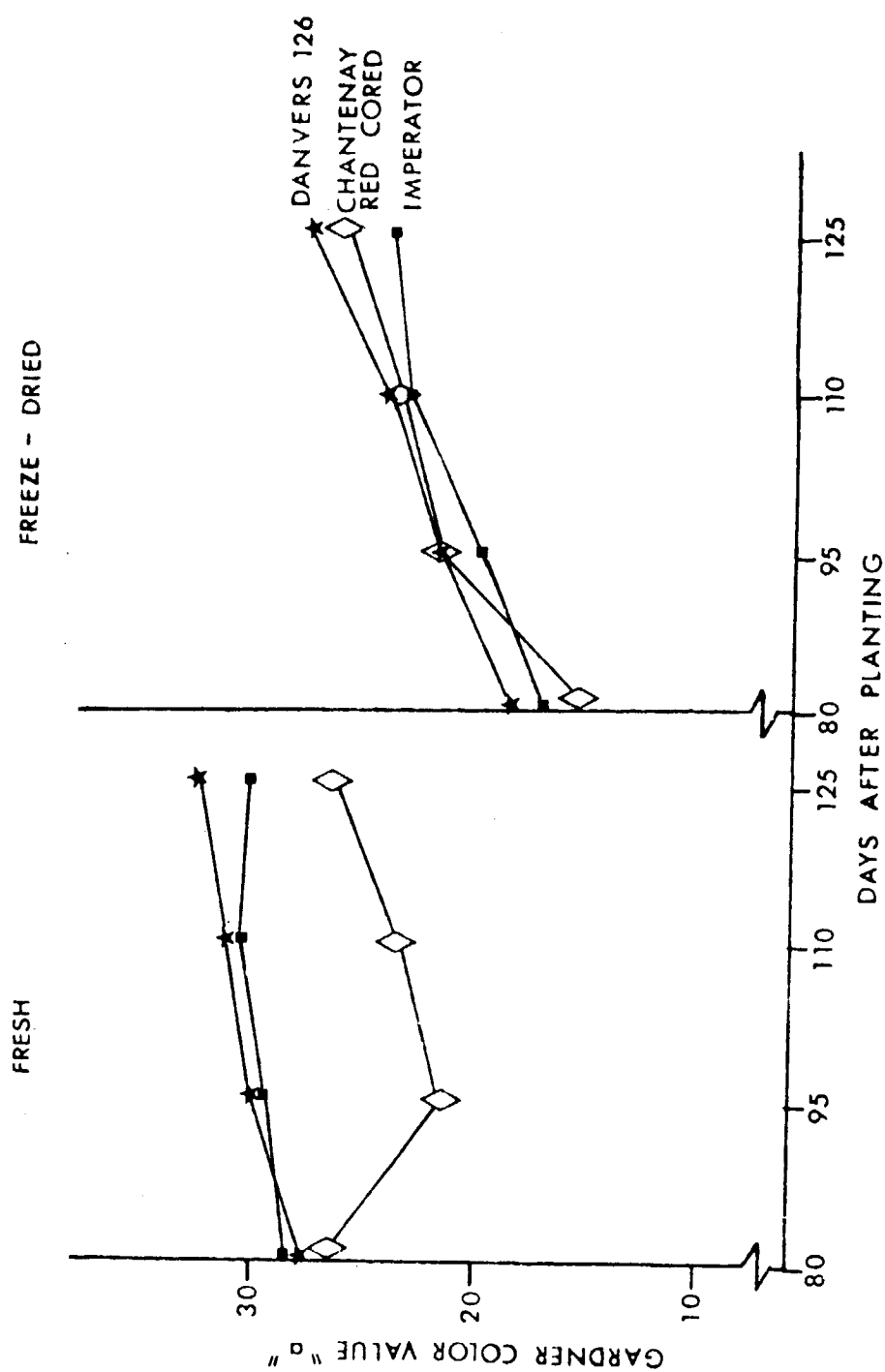


Figure 6. Effect of harvest date on Gardner Color "a" values of fresh and freeze-dried carrots of three cultivars.

Table 3. Effect of harvest date on Gardner color value "b" of fresh and freeze-dried carrots of three cultivars.

Cultivar	Fresh Carrots			Freeze-dried Carrots		
	Days After Planting			Days After Planting		
	80	95	110 125	80	95 110 125	
	Mean Gardner "b" Value			Mean Gardner "b" Value		
Danvers 126	32.3 ^{bcdefg}	32.2 ^{bcdefg}	32.3 ^{bcdefg} 31.4 ^{efg}	29.1 ^h	33.1 ^{bcd}	31.3 ^{fg} 31.0 ^g
Imperator	33.1 ^{bcd}	35.3 ^a	35.1 ^a 31.7 ^{defg}	26.4 ⁱ	33.7 ^b	33.2 ^{bc} 31.2 ^{fg}
Chantenay Red Cored	31.3 ^{fg}	33.5 ^{bc}	32.1 ^{cddefg} 32.5 ^{bcdef}	31.1 ^{fg}	32.8 ^{bcde}	31.6 ^{defg} 30.9 ^g
Standard Deviation	0.90	1.56	1.68 0.53	1.91	0.46	1.02 0.15
Overall Mean	32.2	33.7	33.2 31.9	27.8	33.2	32.0 31.0

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Means are of four determinations.

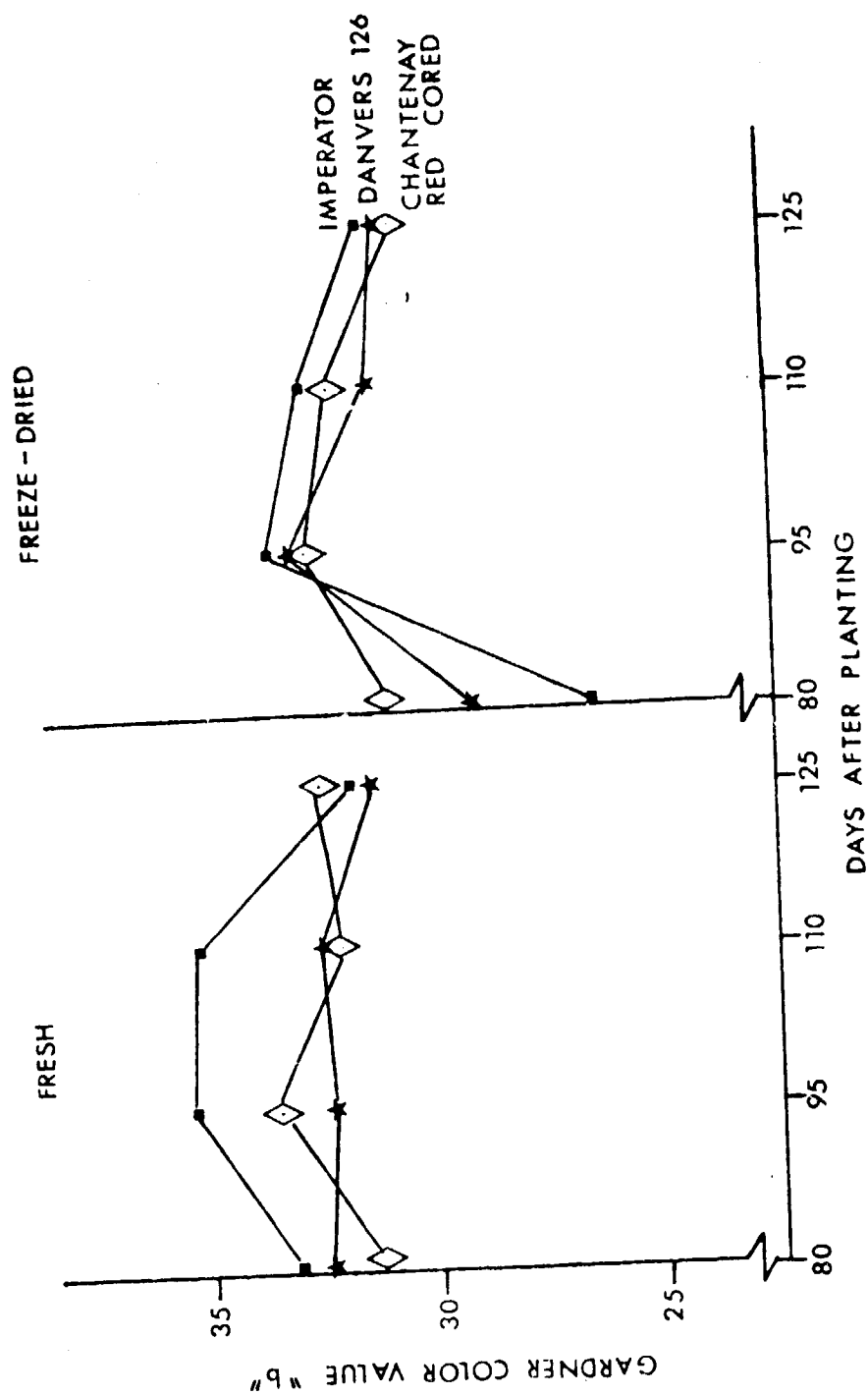


Figure 6a. Effect of harvest date on Gardner Color "b" values of fresh and freeze-dried carrots of three cultivars.

maturity and condition were all shown to be significantly different ($Pr > F = .0001$) for "b" color. Variety, maturity and condition were all shown to have highly significant interaction ($Pr > F = .0001$).

Alcohol Insoluble Solids

The results of the analysis for alcohol insoluble solids are shown in Table 4 and graphically illustrated in Figure 8. The values for fresh carrots ranged from 3.65% for Chantenay Red Cored at 80 days to 3.99% for Imperator at 125 days. The freeze-dried carrots varied from 3.74% for Chantenay Red Cored at 80 days to 4.06% for Danvers 126 at 110 days. The overall mean for alcohol insoluble solids increased slightly with maturity. Imperator and Danvers 126 had the greatest percentage of alcohol insoluble solids while Chantenay Red Cored possessed the least. Alcohol insoluble solids showed a slight increase after processing. This data is in agreement with Bennet (1976) for cellulose and dry matter. The fluctuations in alcohol insoluble solids could reflect changes in cellulose content. Since cellulose is resistant to most processing methods, the increase in cellulose content after processing could reflect a loss of soluble constituents during processing. Hruzek (1973) reported a similar increase in cellulose content after processing.

Analysis of variance for alcohol insoluble solids is shown in Appendix D. Significant differences ($Pr > F = .0026$) for alcohol insoluble solids were found only for varieties.

Viscosity

Effective viscosity was measured in an attempt to establish, with ease of measurement, a relationship between quality of freeze-dried

Table 4. Effect of harvest date on alcohol insoluble solids of fresh and freeze-dried carrots of three cultivars.

Cultivar	Fresh Carrots			Freeze-dried Carrots		
	Days After Planting			Days After Planting		
	80	95	110	80	95	110
	Percent Alcohol Insoluble Solids			Percent Alcohol Insoluble Solids		
Danvers 126	3.82 ^{cdef}	3.87 ^{bcdef}	3.95 ^{abcd}	3.86 ^{bcdef}	3.91 ^{abcde}	4.06 ^a
Imperator	3.87 ^{bcdef}	3.88 ^{bcdef}	3.99 ^{abc}	3.89 ^{abcdef}	3.95 ^{abcd}	4.01 ^{ab}
Chantenay Red Cored	3.65 ^g	3.72 ^{fg}	3.81 ^{defg}	3.79 ^{defg}	3.74 ^{fg}	3.84 ^{bcdef}
Standard Deviation	0.12	0.09	0.09	0.05	0.09	0.12
Overall Mean	3.78	3.82	3.92	3.85	3.87	3.97
					0.05	0.05

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Means are of four determinations.

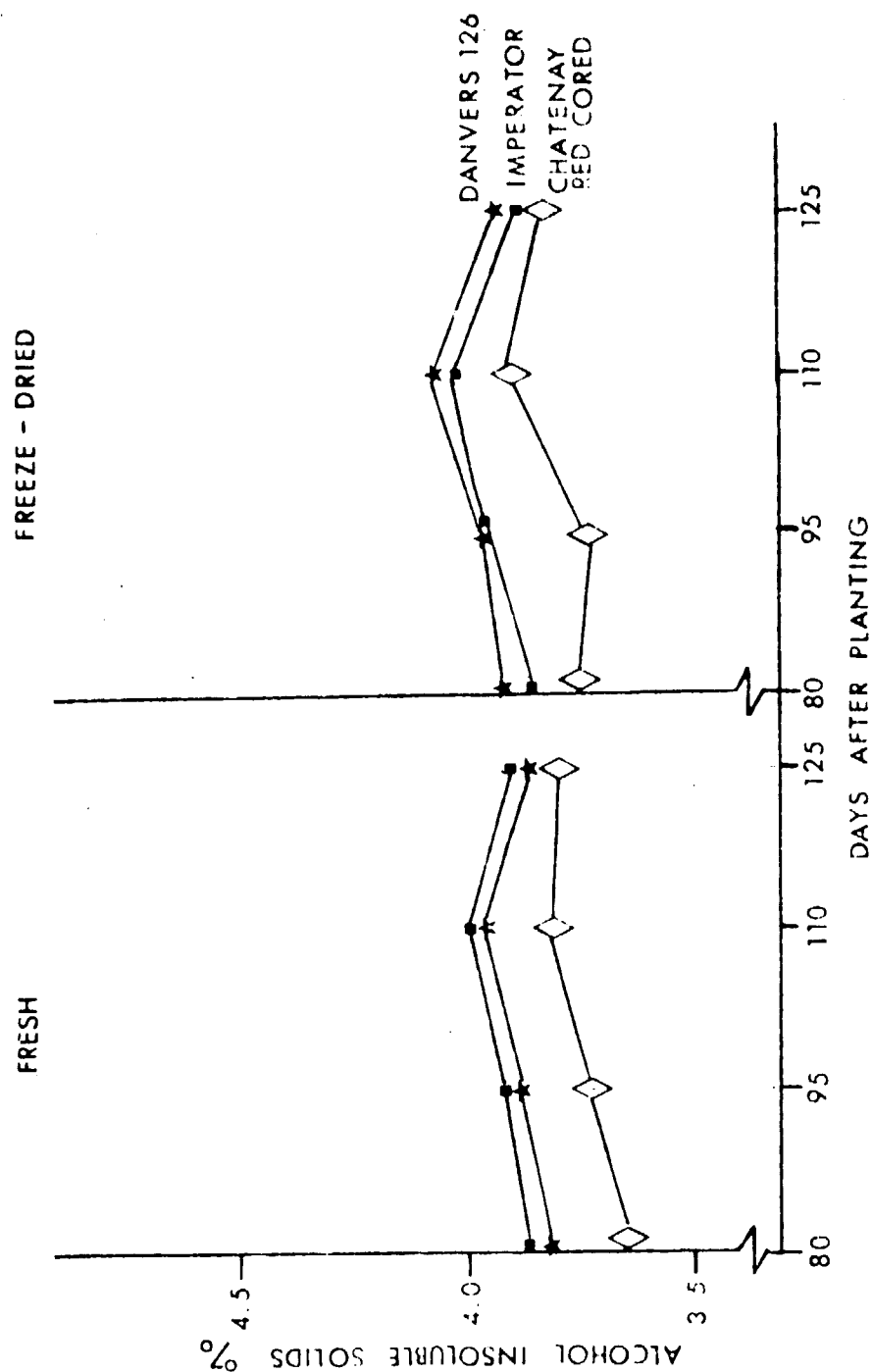


Figure 6b. Effect of harvest date on alcohol insoluble solids of fresh and freeze-dried carrots of three cultivars.

carrots and a rheological parameter. The results of viscosity measurement are presented in Table 5. The values ranged from 14.89 poise for Danvers 126 at 80 days to 23.67 poise for Danvers 126 at 110 days. The increase in viscosity with maturity follows the findings of Bennet (1976) for dry matter. The increase in viscosity may be due to a combination of cellulose and sugar changes during maturation.

Analysis of variance for viscosity is shown in Appendix E. Significant differences for viscosity were found only in maturity ($Pr > F = .0052$).

Core/Cortex Ratio

The results of core/cortex measurements are presented in Table 6. The lowest value was 3.16 for Chantenay Red Cored at 125 days and the greatest was 4.53 for Imperator at 95 days. The ratio remained fairly constant throughout the growing period within varieties. Analysis of variance for core/cortex ratio is shown in Appendix F. Significant differences were shown between varieties ($Pr > F = .0223$). Chantenay Red Cored had the lowest overall ratio at 3.46 while Imperator was highest at 3.97.

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PART II

THE EFFECT OF MICROWAVE
BLANCHING ON THE NUTRITIONAL
AND TEXTURAL QUALITY OF
FREEZE-DRIED SPINACH.

REVIEW OF LITERATURE

Spinach (Spinach oleracea) is a representative of Chenopodiaceae family. Though essentially a cool-season crop, the culture of spinach is possible during the winter where the weather is mild; elsewhere it is grown as a spring and fall crop (Bailey and Bailey, 1976).

Leaf-type, such as smooth, savoy, or semi-savoy, was not found to be critical in producing compressed, freeze-dried spinach. The color of spinach in the final product is most dependent on the blanching method used (Wisakowsky, 1975).

Nutritionally, spinach has a high protein, mineral and vitamin content as compared to other vegetables. A 100 g serving provides 20% of the Recommended Dietary Allowance for iron, twice the amount of vitamin A, 65% of the vitamin C and is rich in B vitamins (Robinson and Lawton, 1977). By proximate analysis, spinach is found to contain 91.3% water, 2.3% protein, .3% fat, 3.2% carbohydrate, 1.9% fiber, and 2.3% ash (Winton, 1935).

The nutrient content of freshly harvested plants varies. These variations result from an interplay of factors such as: genetics, climate, soils, soil fertility, and maturity. After harvesting, vegetables are physiologically active. Enzymatic and respiratory activities continue bringing about profound changes unless controlled. During storage periods, the commodity may undergo deterioration in nutritive value, particularly the destruction of ascorbic acid. The extent of this destruction is dependent on the length of time and temperature. Kelly et al. (1950) found a slight loss in provitamin A in spinach held for 37 hr at room temperature, and a 30% loss when

held for 133 hr in cold storage. Nutrients are destroyed when foods are processed largely because they are sensitive to the pH of the liquid media, to oxygen, light and heat or combinations of these processes (Harris and Karmas, 1975).

Freeze Drying

Freeze drying is the removal of water as a vapor from a frozen substance. The water is vaporized from the frozen state without passing through the liquid phase, a process also known as sublimation or lyophilization. The temperature of the sublimation zone in the material being freeze dried must be held below the triple point temperature of the aqueous solution in the material being dried.

As a method of dehydration, freeze drying produces the highest quality product obtainable. Reasons include the porous, rigid, non-shrunken structure in the dried product which, in turn, favors rapid rehydration. Other benefits of freeze drying include low processing temperature, the relative absence of liquid water and the rapid transition of the material from a fully hydrated to nearly completely dehydrated state. This minimizes degradative reactions such as non-enzymatic browning, protein denaturation and enzymatic reactions (King, 1971).

While freezing is a general prerequisite to freeze drying, the operations preliminary to freezing are essentially product oriented. Items with relatively impermeable skins such as peas are scarified to facilitate escape of water vapor. Vegetables are blanched to inactivate enzymes, set starch and color, and to remove earthy odors (Brockman, 1974).

Freeze-dried products undergo no important loss of vitamins as a result of freeze drying (Thomas and Calloway, 1961); nor is the nutritive quality of the proteins significantly changed (deGroot, 1963). The major loss of acceptability occurs during storage at elevated temperatures and in pre-processing treatments such as blanching. Oxidative changes are common, especially in the case of carotenoid pigments which are vulnerable to gaseous oxygen (Brockman, 1974).

The growth of the freeze-dried food industry depends on the interactions among the unique properties of freeze-dried products, their acceptance, their negative features, and the economic realities of their production. Even though freeze-dried products are of very high quality, it remains an expensive process (Brockman, 1974; Wisakowsky, 1975).

Blanching

Blanching is the process which precedes the freezing of vegetables wherein heat is used to inactivate certain enzyme systems that contribute to undesirable changes in flavor, color, and aroma, as well as nutritive value during storage. Blanching also serves to deaerate, tenderize and soften the texture of foods. Conventionally, the process is carried out with steam or water. Disadvantages to these methods include textural damage, leaching of water-soluble vitamins, and an effluent high in biological oxygen demand (B.O.D.). The criterion for evaluating the adequacy of the blanching operation is enzyme inactivation (Decareau, 1972).

Peroxidase is one of the more heat-stable enzymes in plants

(Reed, 1966). Inactivation of this enzyme is often used to determine when the vegetable has been satisfactorily blanched (Chen et al., 1971).

Microwave Blanching

Microwaves cause the molecules within the food material to align themselves with the direction of the electrical field and oscillate around their axis. This oscillation creates intermolecular friction resulting in heat (Aref, 1968). The penetrating nature of microwaves provides a means of deep heating a material without relying on a temperature gradient. This energy can be concentrated to effect a very rapid heating and because water is a particularly high absorber of microwave energy, it is especially applicable to the heating of foods (Huxsoll et al., 1970).

The conservation of energy is a concern of the food processor. Hirst (1974) estimated that the food cycle used about 12% of the total U.S. energy. Of this amount, food processing accounts for approximately 50%. Recently, the price of microwave generators has dropped from \$1.40 per watt to \$.35, a seventy-five percent decrease, and that price is expected to further decline (Butler, 1979).

Microwave heating has been applied to the blanching of food products. Since it can be assumed that microwave energy has no direct enhancing effect on the degradation of food components other than through temperature elevation, microwave blanching should result in nutrient retentions at least equal to that achieved during steam blanching and better than that achieved during water blanching (Harris and Karmas, 1975).

The influence of 3,000 mc radiation (radar) blanching on spinach, broccoli, carrots, peas, and green beans was investigated by Proctor and Goldblith (1948). The vegetables were packed in plastic bags and heated 20-30 sec with radar. This was sufficient to inactivate catalase and peroxidase. They were then cooled by placing the bags in cold water. Under these conditions which avoided contact with water, ascorbic acid retention was essentially 100%. In contrast, ascorbic acid retention was 37-100% for steam and 24-93% for water blanching. The largest losses of ascorbic acid were for spinach. The decrease in ascorbic acid during water or steam blanching resulted from leaching rather than from destruction. The advantage of radar blanching was protection from water while the vegetables were hot. Broccoli blanched in the microwave was higher in ascorbic acid retention (79.22%) than that blanched in water at 100° or 77°C. During blanching in the microwave, volatile acids and water vapor from the broccoli were thought to be condensed by the plastic bag. The cooked broccoli blanched by this method was spotted in appearance (Eheart, 1967).

Rapid heating of plant tissues to completely inactivate enzymes should be favorable in retaining ascorbic acid in blanched food. Using 100 g samples of fresh vegetables, the optimum periods for enzyme inactivation (blanching) were determined to be 30 seconds or less (Proctor and Goldblith, 1948).

Dietrich et al. (1970) compared microwave, steam, and water blanching and verified that microwave blanching resulted in better ascorbic acid retention in brussel sprouts; however, the best product was achieved with combination processes involving microwave and water

blanching procedures. The microwave treatment gave rapid heat input into the product. A holding period in hot water following microwave treatment allowed for thermal equilibration.

Vitamins

The blanching operation can significantly reduce the nutrient content of the food, the extent being dependent on the blanch method and product. In vegetables, both the amount of water used and the cooking time were factors found to affect the retention of vitamins (Lorenz, 1976).

Optimization of the blanching process with respect to nutrient retention can be assessed by considering leaching and oxidative losses. The two traditional methods of blanching use either hot water or steam as the heat transfer medium. For water blanching, the loss of water-soluble vitamins increases with contact time and fat-soluble vitamins are relatively unaffected. A decrease in soluble solids also occurs (Fang et al., 1971). This was explained by leaching of water soluble solids into the blanch water when the cells were disrupted by the heat treatment. Steam blanching has the disadvantage of over-blanching the particles in the periphery of the tray; while the particles in the

The lipid soluble carotenes found in plant material are precursors to vitamin A. In deep green vegetables such as spinach, the carotenes are masked by chlorophyll. Upon hydrolysis, each molecule of beta-carotene theoretically yields two molecules of vitamin A. Under an inert atmosphere they are stable; but when heated in the presence of oxygen, and especially at higher

temperatures, the carotenes rapidly lose activity. When cooked, losses range from 0 to 40% (Robinson and Lawler, 1977; Harris and Von Loesecke, 1973). The structure of beta-carotene is shown in Figure 7.

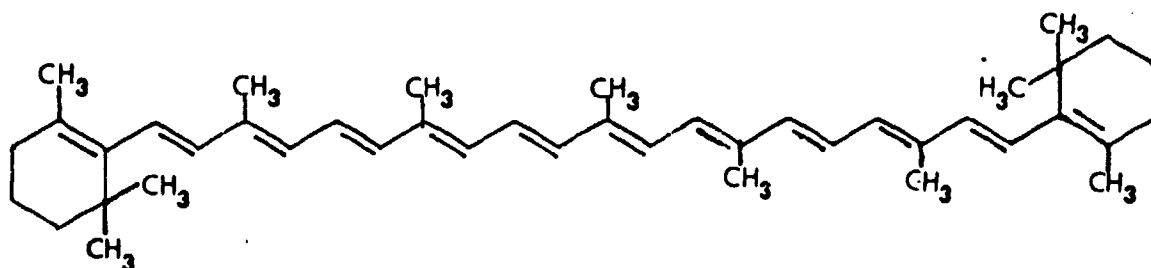


Figure 7. Structure of beta-carotene.

Vitamin E is not a single entity but a group of structurally similar compounds known as tocopherols. The most common of these and the one found in green leafy vegetables is alpha-tocopherol (Figure 8).

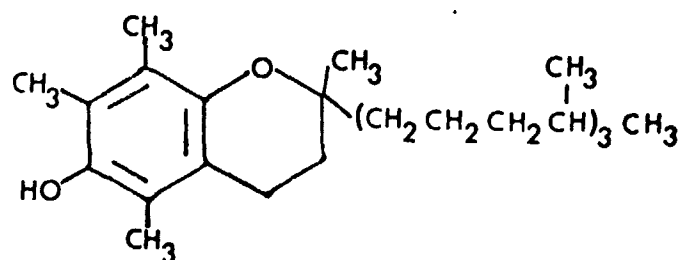


Figure 8. Structure of alpha-tocopherol.

High temperatures and acids do not affect the stability of vitamin E; but oxidation takes place readily in the presence of rancid fats, lead or iron salts. Decomposition occurs in ultraviolet light. Vitamin E acts as an antioxidant. By accepting oxygen, vitamin E helps to prevent the oxidation of vitamin A and ascorbic acid thereby sparing these vitamins (Robinson and Lawler, 1977).

Of all the vitamins, ascorbic acid is the most easily destroyed (Figure 9). It is highly soluble in water. The oxidation of ascorbic acid is accelerated by heat, light, alkalies, oxidative enzymes, and traces of copper and iron.

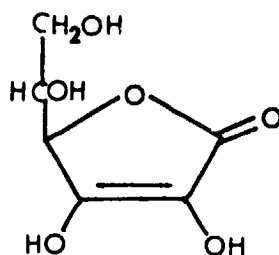


Figure 9. Structure of L-ascorbic acid.

High Pressure Liquid Chromatography

Around 1906, Tsweet discovered the technique of chromatography for the purpose of separating plant pigments by elution through a column of absorbent. Separation arose because of the different affinities of substances for the absorbent with which the column was packed. In 1941, Martin and Synge developed liquid-liquid (partition) chromatography. The sample components partitioned themselves between the two liquid phases according to solubility (Done et al., 1974).

More recently, thin-layer and paper chromatography have been used for rapid analyses. In 1952, Martin and Janes developed gas chromatography, a sophisticated technique especially suited for mixtures of gases, volatile liquids and solids (Bauer et al., 1978).

Thin-layer chromatography (TLC) is the most widely used method for separating vitamin E isomers. Gas chromatography has also been employed as an analytical method after preliminary separation and clean-up of the samples by preparative TLC. Since tocopherols are antioxidants and

light sensitive, the TLC system has the disadvantage of leaving them vulnerable during the time they are on the plate. Gas chromatography requires derivatization prior to chromatography, making the isolation of pure tocopherols difficult (Cavins and Inglett, 1974).

Modern high pressure liquid chromatography (HPLC) is particularly well suited to the rapid separation of vitamins and possesses many advantages. Speed, specificity, detection of lower potency levels, and simplified sample workups have all been demonstrated as feasible for HPLC (Conrad, 1975). Since analyses are performed at room temperature, the destructive high temperatures needed for volatilization in gas chromatography (GC) are eliminated and degradation products from this procedure are not encountered. Sample cleanup and preparation can be reduced significantly; extracts can often be injected directly. Judicious choice of column packing and solvent combination may allow both fat and water-soluble vitamin determinations on the same column (Conrad, 1975).

HPLC dates from the late 1960's with the last ten years seeing the rapid evolution of techniques and instrumentation toward efficiencies comparable to those of GC. The major components of the HPLC system include a solvent reservoir, high pressure pump, injector system, column and detector (Figure 10). The heart of the LC system is the column. A wide variety of column packings and the broad range of solvents offer numerous approaches to effect a separation. For rapid and direct multi-vitamin assays a "reverse phase" packing consisting of long-chain hydrocarbons chemically bonded to the surface is particularly efficient. Reverse-phase partition chromatography was found to be

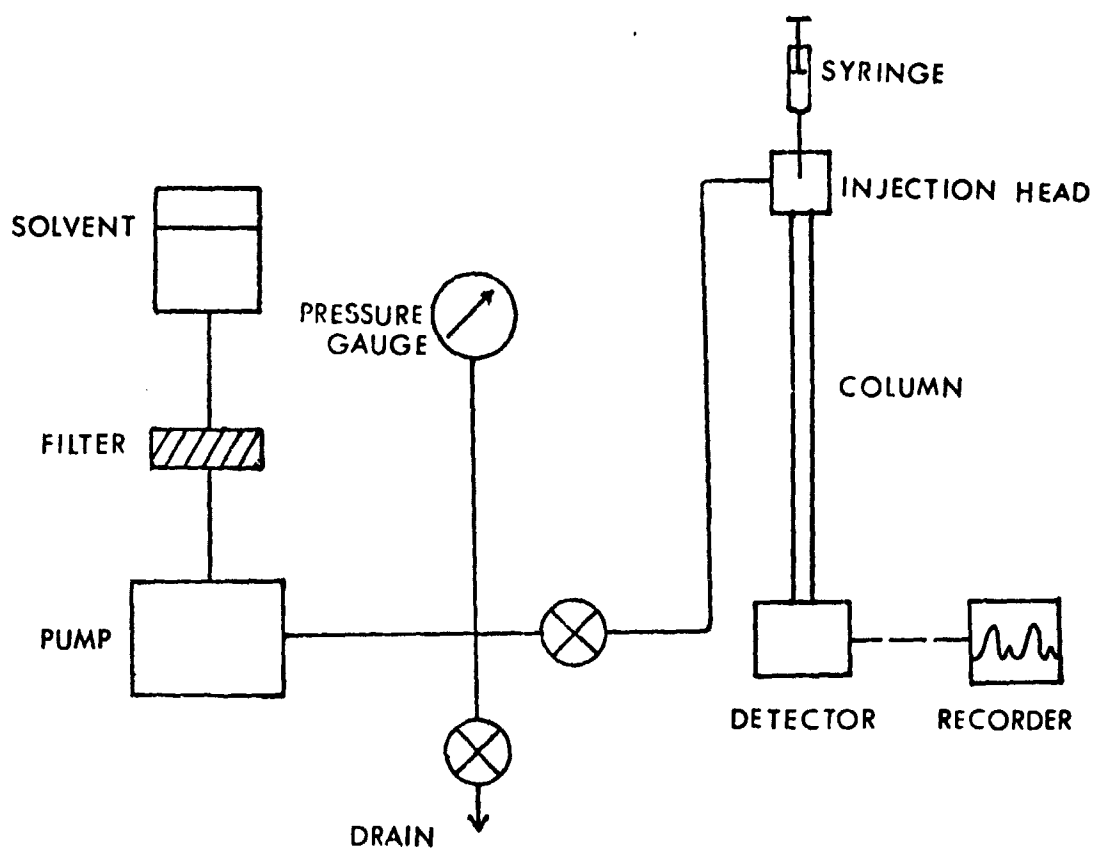
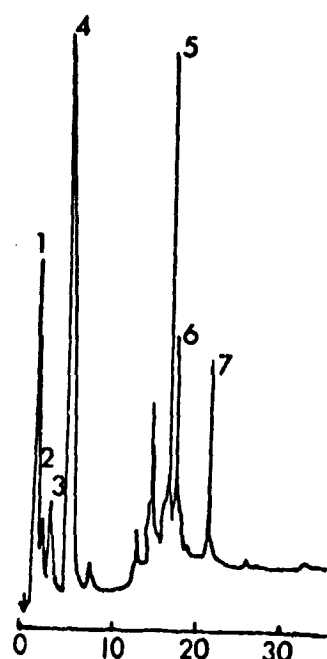


Figure 10. Outline of a high pressure liquid chromatograph.

best for the separation of the fat-soluble vitamins in studies by Williams et al. (1972). Essentially, the method consists of the partition of relatively non-polar molecules, such as the fat-soluble vitamins, between a polar mobile phase and a non-polar stationary phase. For these separations a water/alcohol solvent is usually used as the mobile phase. The fat-soluble vitamins and their esters vary considerably in chromatographic behavior and may require different stationary or mobile phases for satisfactory separation (Williams et al., 1972).

The fat-soluble vitamins are often found in matrices of fat or oil. An example of this is vitamin A in cod liver oil. The high molecular weight triglycerides in cod liver oil are not soluble in the water/alcohol mobile phases used with reversed-phase and they precipitate on the column. Williams et al., (1972) found that this precipitate can coat the column packing and cause a tailing sample front. The most common solution to this problem is the removal of triglycerides from the vitamin sample before chromatography. Various methods for this exist including saponification. They used purified samples which did not require prior saponification and they did not encounter any interfering substances typically present in food material. Head and Gibbs (1977) found that extracting food composite samples prior to saponification was necessary to separate the pulp from the lipid material.

Chromatographic conditions can be adjusted to suit the special requirements of a particular vitamin assay. If a formulation is composed of only one vitamin, then the analysis can be achieved using a mobile phase at some appropriate constant composition, thereby reducing the analysis time. A typical high pressure liquid chromatograph for vitamin standards is presented in Figure 11.



PEAK IDENTITY

- 1 Riboflavin $C_{17}H_{20}N_4O_6$
 2 Vitamin B₁₂ $C_{63}H_{88}CoN_{14}O_{14}P$
 3 Vitamin K₃ $C_{11}H_8O_2$
 4 Vitamin C $C_6H_8O_6$
 5 Vitamin D₂ $C_{28}H_{44}O$
 6 Vitamin E $C_{29}H_{50}O_2$
 7 Vitamin A $C_{20}H_{30}O$

OPERATING CONDITIONS

Column: ODS "Permaphase"

Column Temperature: 50°C

Linear Gradient: From 100% H₂O to 100% CH₃OH
 at 3% per minute

Column Pressure: 900 psi

Flow Rate: 0.9 cc/minute

Detector: UV Photometer

Figure 11. High pressure liquid chromatograph of vitamin standards (Schmit et al., 1971).

Histology and Texture

The origin of textural quality in fresh and processed vegetables is essentially histological. Freeze-dried vegetables are of porous structure which allows rehydration at a faster rate than air-dried vegetables. However, because of blanching and freezing prior to freeze drying, texture after rehydration is adversely affected; rehydrated products possess a less firm texture than their fresh counter-parts. The physical properties reflecting turgidity depend largely upon the cell wall structure in plant tissues. The claim is made that tissue softening is due primarily to the easy separation of the cells and secondarily to the loss of rigidity in the individual cell walls (Sterling, 1955). All parenchymatous fruit and vegetable tissues undergo changes in intercellular pectic substances during heating and the resulting decrease in firmness relates to the formation of soluble pectins. This decrease in firmness occurs at high and low pH levels (Reeve, 1970). During processing, a loss in cell rigidity occurs upon death of the cell and destruction of its organized cytoplasmic membranes (Shimazu and Sterling, 1961). The loss in moisture retaining properties of the cellular membrane results in loss of turgor (Mohr, 1974). Another important change that occurs in the heating of fruit tissues is an expansion and escape of intercellular gases which further separates the intact cells (Reeve and Leinbach, 1953).

Commercially freeze-dried foods are not only heat treated by blanching but are also frozen at temperatures ranging from -18 to -35°C before being dehydrated. This indicates that further cell changes due to freezing may be expected. Certain aspects of the effects on textural

qualities are closely related to the structural differentiation of specialized tissues. Thick-walled tissues are naturally resistant; as they are usually more compact and their cells are of much smaller diameter than the surrounding large-celled parenchyma. Localized differential rates of freezing often result in torn parenchyma cell walls and the development of large voids. Mohr (1974) found that tissues of low starch and high moisture such as spinach leaves become very limp in appearance upon thawing yet their subcellular components remained structurally intact. Typical freeze-thaw damage to fine structure included the rupture of the vacuole membrane, some displacement of cytoplasmic components; peripheral clumping of nuclear material and complete fragmentation of most of the endoplasmic reticulum, ribosomes, and mitochondria. Whether the freezing stage was carried out slowly (1 1/2 minutes to 8 minutes) or rapidly made no apparent difference in protoplasmic disruption. Blanching caused the near complete destruction of fine structure underlining the sensitivity of the spinach tissue to heat. Any subsequent effect due to freezing and thawing proved insignificant in comparison. According to Reeve (1970), cytological changes most often manifest themselves as loss of characteristic texture, a cracking of the brittle frozen food, and a drippage of cell constituents upon thawing and holding.

Scanning Electron Microscopy

With the advent of the scanning electron microscope (SEM) a new dimension was added to histological study. Such micrographs provide a realistic three-dimensional quality with great depth of field and clarity of detail. As the electron beam strikes the surface of the

specimen, secondary electrons are emitted producing an image. Sample size and thickness are limited only by the capacity of the instrument's evacuated specimen chamber (Everhart and Hayes, 1973).

Histological observation of freeze-dried spinach is further facilitated since the sample is directly observed in the freeze-dried state without proceeding through an embedding process.

The various types of microscopy complement each other rather than compete. Each provides unique information about the microstructure of the plant material.

The Need

Microwave blanching has been shown to be economically feasible with nutritional and processing advantages. However, the absorption of microwave energy by the plant tissue can be assumed to result in chemical and physical changes as yet unexplained. The intermolecular friction from microwave heating may result in internal cell pressure leading to rupture, a loss of cell contents and organization.

Differences in vitamin retention may be understood in terms of lipid/water solubilities; however, oxidation and related degradative reactions may be caused by the rapid heat onset.

Surface and internal damage from microwaves oscillating within the individual tissue cells may be expected and such would conceivably result in textural variation. The extent of damage will affect the efficiency of the freeze drying process as well as rehydration rates, and final product quality.

The goal of this study was to explain vitamin interrelationships, anatomical changes, and oxidative deteriorations in terms of

preprocessing microwave treatments. Statistical methods were employed in the gathering of data and interpretation of results.

MATERIALS AND METHODS

Production and Processing

Commercially grown spinach of the Norgreen cultivar was used for this study. The spinach was harvested in the late Spring of 1979, two days prior to arrival at the Adriance Laboratory, College Station, Texas. Upon arrival, the spinach leaves were washed and trimmed of the petiole and all yellow portions. The washed leaves were torn into 1 to 2 inch segments and split through the center vein to facilitate enzyme deactivation during blanching.

The samples were either steam, water, or microwave blanched for a period sufficient to inactivate enzymes. For water blanch treatment, samples of 200 g were tied in cheesecloth and immersed in approximately 3000 ml of water at 100°C for 2 minutes. Samples for steam blanching were spread on a wire tray and subjected to steam in a partially closed retort vessel for 2 minutes. Immediately after water or steam blanching the samples were immersed in ice water. For microwave blanching, samples of 100 g were placed in non-rigid, thermoplastic bags. The bags were suspended in the oven from the tip of an inverted glass funnel for the most efficient heating as described by Proctor and Goldblith (1948). Enzyme inactivation was accomplished with a heat exposure 1 minute and 35 seconds. A Litton 420 microwave oven with a power rating of 650 watts was used for heating. An ice water quench stopped the blanch, and caused an immediate shrinkage of the bag around the product expelling entrapped air. The spinach never came into direct contact with water.

Freeze drying was accomplished using a model REPP sublimator,

manufactured by the Virtis Company, Gardiner, New York. The samples were freeze dried at a condenser setting of -45°C and a shelf temperature of 27°C . The finished freeze-dried product was vacuum sealed in #303 cans and stored at -40°C until subsequent evaluation.

Moisture content of the fresh spinach was determined by drying samples to a constant weight. Samples of finely shredded leaves were weighed into tared aluminum dishes. These samples were dried under vacuum at 100°C for 20 hr.

Evaluation

Evaluation was conducted on freeze-dried spinach leaf tissue to compare the effects of microwave versus conventional blanching. Triplicate samples were utilized in each analysis.

Catalase-Peroxidase

A negative catalase-peroxidase endpoint was used to determine adequacy of blanch (Huffman et al., 1977). In an effort for uniform heat penetration, samples of single layer thickness were used in all three blanch methodologies: steam, water and microwave.

Color

Color was measured with a Gardner Automatic Color Difference Meter Model XL-10A, calibrated with the Gardner Color Standard Plate #C-DG-1481-59. Following the method of Wisakowsky (1975), readings were taken on a 1:1 puree of spinach and water. For comparison, fresh and cooked samples were included. The purees were placed in an

optically correct cup to a depth of 3/4 inches. Measurement was in terms of Gardner Color values of "L", "a" and "b". The "L" is considered to be a measure of lightness of sample color; "+a" a measure of redness; "-a" a measure of greenness; "+b" a measure of yellowness; and "-b" a measure of blueness (Francis and Clydesdale, 1969).

Carotene

Carotene analysis was done by the procedure outlined in Methods of Vitamin Assay (Anonymous, 1966). Samples were ground through a 40-mesh screen. A 0.5g sample was extracted using an acetone-petroleum ether mixture (30:70 parts by volume) for one hour on a Goldfish extractor. The extracts were allowed to cool before being condensed over steam to approximately 10 ml.

Separation was accomplished on a magnesium-super cel (1:3) column. The extract was eluted with 3% acetone in petroleum ether. The eluent was collected until the carotene band had moved off the column and the filtrate was colorless. The eluent was diluted to 500 ml and read at 436 nm on a Bausch and Lomb Spectronic 20 Spectrophotometer. Efficiency of recovery was found to be approximately 98% when tested using samples of known beta-carotene amounts. Since beta-carotene isomerizes readily during heating to give neo-beta-carotene and since neo-beta-carotene has been found to occur naturally in some foods, a more accurate value of total carotene was obtained by reading at 436 nm. Absorbance was used to calculate total carotene as beta-carotene as justified in the methodology (1966). A standard curve was measured using pure beta-carotene supplied by the Aldrich Chemical Co., Inc., Milwaukee, Wis.

Ascorbic Acid

A modification of the microfluorometric method of Duetsch and Weeks (1965) was used for vitamin C assay. This method is based on the reaction of dehydroascorbic acid with o-phenylenediamine to give a fluorescent quinoxaline. The development of the fluorescent derivative of the vitamin is prevented by forming a boric acid-dehydroascorbic acid complex prior to addition of the diamine solution. This provides a means of differentiating between the fluorescence from the vitamin and that from possible interfering substances.

Two and a half grams of sample were blended with 100 ml metaphosphoric-acetic acid solution until homogenized and then filtered through Norite. Five ml each of filtrate and NaOAc were combined and diluted to 100 ml with water. Fluorescence was measured on an Aminco SPF-500 Series Ratio Spectrofluorometer with a Linear Instrument Corp. Model 285/MM recorder. Emission setting was at 430 nm with a bandpass of 40; excitation setting was 350 nm with a bandpass of 4. A standard calibration curve was prepared using reagent-grade L-ascorbic acid supplied by Fisher Chemical Co., Fair Lawn, New Jersey.

Alpha-Tocopherol

High pressure liquid chromatography was employed for alpha-tocopherol separation. Following the suggestion of Conrad (1975), a modification of the AOAC method (1975) was used to form a saponified spinach extract prior to injection. One gram samples were extracted in ethanol for four hours using a Goldfish apparatus. The extract was washed with water and petroleum ether, discarding the aqueous layer.

The petroleum ether was evaporated off under nitrogen and the extract was saponified with concentrated KOH. The unsaponifiable material was washed with water till neutral and concentrated under nitrogen to remove the ether. Samples were transferred with methanol, brought to 25 ml volume, and stored at -40°C until chromatographed.

An Altex Model 312 MP Programable Liquid Chromatographic system equipped with an UV detector and 10MV linear recorder was employed for separation. A stainless steel LiChrosorb C_{18} (reverse phase) column, 3.2 x 250 mm, 10 μm particle size was used. Conditions of operation included: 95% MeOH mobile phase at a flow rate of 2 ml/min; sample size was 20 μl and recorder sensitivity was .01 AUFS. A mobile phase of constant composition as compared to one of gradient elution was found to allow faster separation with reproducible results. A 95:5 MeOH/ H_2O mobile phase yielded better resolution than a 100% MeOH phase without the sacrifice of speed. The sample was drawn through glass wool into the syringe prior to injecting.

Alpha-tocopherol identity was determined through peak location of a purified sample of alpha-tocopherol. Quantitative determinations were then made using a standard dilution series plotting peak area against concentration.

Rehydration

A weighed sample of the freeze-dried leaves was immersed in distilled, deionized water at 60°C . After one minute hydration, the spinach was drained for five minutes through a wire mesh strainer, blotted free of excess water and reweighed. Rehydration ratios were calculated by dividing the rehydrated weight by the initial freeze-dried

weight (Wisakowsky, 1975).

Texture

Texture was determined using an Ottawa Texture Measuring Cell system (O.T.M.S.). Rehydrated samples of 7 g were placed in the load cell. An Instron Universal Testing Machine, Model 1122, manufactured by the Instron Corp., Canton, Massachusetts was used to supply an axial compression load with a constant load rate of 50 mm/minute. A force deformation curve was obtained (Figure 12). Maximum force (Newtons) obtained was used as an index of texture (Voisey et al., 1972).

Scanning Electronmicroscopy

Histological examination of the spinach tissue was conducted on a JSM-U3 scanning electron microscope manufactured by the Japanese Electron Optical Laboratory. Tissues were examined in the freeze-dried state and after 1 minute rehydration. Samples were coated with pure gold in an argon atmosphere. This preparation was necessary to prevent surface charging of the sample material, thus allowing quality pictures.

Light Microscopy

Fresh, blanched, and freeze-dried tissues were placed in FAA, cytological fixative. This solution is composed of 70% ethanol, formaldehyde, and glacial acetic acid (90:5:5 by volume). The FAA stops the life process of the tissue immediately and preserves the specimen in a life-like manner. After 24 hours, the tissues were transferred

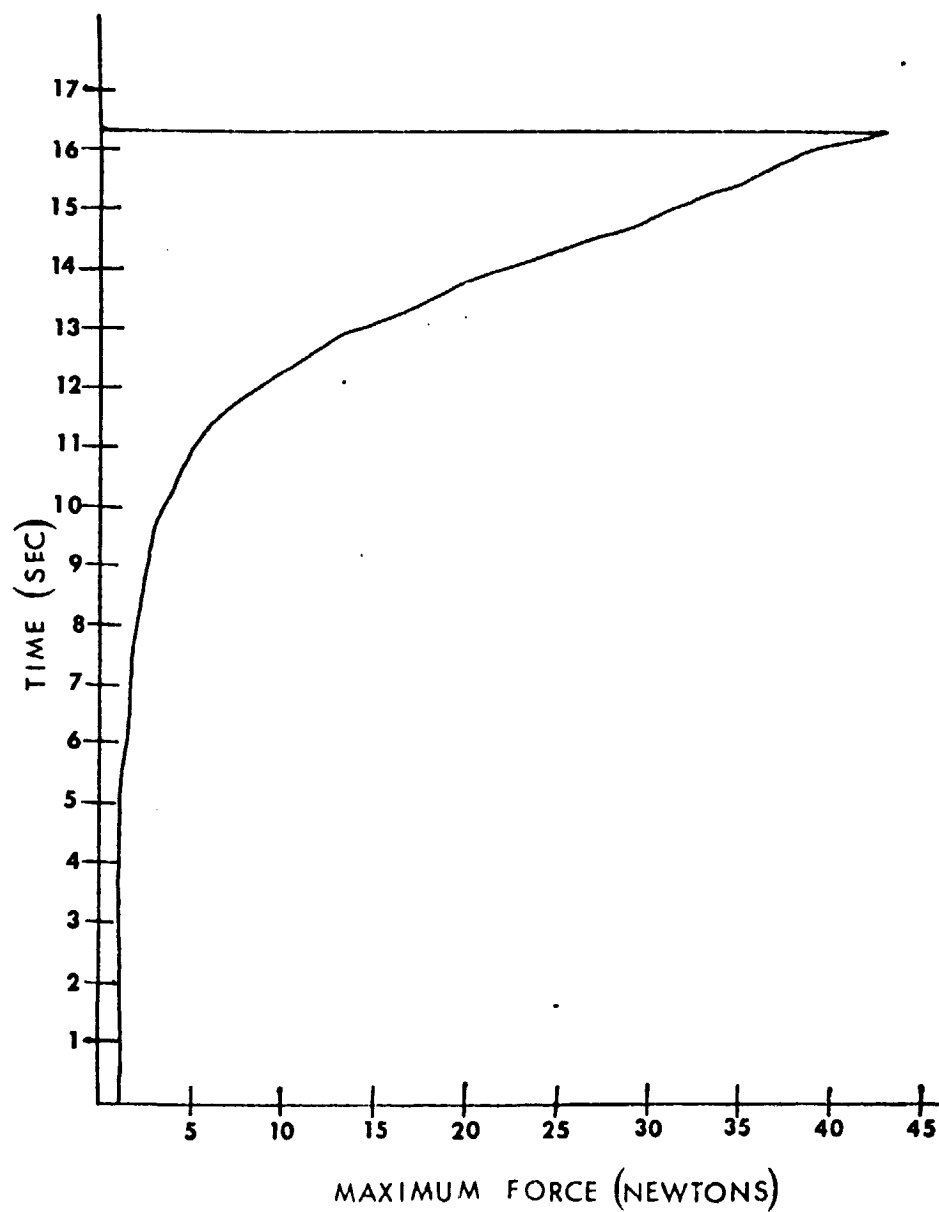


Figure 12. Typical O.T.M.S. cell curve for rehydrated, freeze-dried spinach.

to 70% ethanol. The tertiary-butyl alcohol dehydration series of Bashaw (1976) was followed. A Tissue-Tek II Tissue Embedding Center was used to paraffin embed the samples. Continuous serial sections were generated on a Spencer rotary microtome and affixed to glass slides. These were stained following the safranin-fast green staining schedule (Bashaw, 1976).

Sensory Evaluation

A trained sensory panel composed of eight members utilized a nine point hedonic scale for scoring color, odor, flavor, texture, and appearance of the variously blanched and rehydrated freeze-dried samples. Spinach cooked for 7 minutes was used as an unmarked control. The score sheet used is shown in Figure 13.

Statistical Interpretation

Data from all the tests were examined using analysis of variance to determine differences in blanch methodology. Duncan's multiple range tests were performed to determine differences between treatment means. Pearson correlation coefficients were calculated to indicate relationships between the measure of quality.

TECHNOLOGICAL EXAMINATION									
PRODUCT:								DATE	
TESTERS NAME:									
COLOR									
ODOR									
FLAVOR									
TEXTURE									
APPEARANCE									
Sample Number	Extremely Poor	Very Poor	Poor	Below Fair Above Poor	Fair	Below Good Above Fair	Good	Very Good	Excellent
COLOR									
ODOR									
FLAVOR									
TEXTURE									
APPEARANCE									
Sample Number	Extremely Poor	Very Poor	Poor	Below Fair Above Poor	Fair	Below Good Above Fair	Good	Very Good	Excellent
COLOR									
ODOR									
FLAVOR									
TEXTURE									
APPEARANCE									
Sample Number	Extremely Poor	Very Poor	Poor	Below Fair Above Poor	Fair	Below Good Above Fair	Good	Very Good	Excellent
COLOR									
ODOR									
FLAVOR									
TEXTURE									
APPEARANCE									
Sample Number	Extremely Poor	Very Poor	Poor	Below Fair Above Poor	Fair	Below Good Above Fair	Good	Very Good	Excellent
COLOR									
ODOR									
FLAVOR									
TEXTURE									
APPEARANCE									
Sample Number	Extremely Poor	Very Poor	Poor	Below Fair Above Poor	Fair	Below Good Above Fair	Good	Very Good	Excellent

Figure 13. Sensory score sheet used to evaluate freeze-dried spinach.

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RESULTS AND DISCUSSION

Gardner Color

The mean Gardner color "L" values for fresh, cooked control, and freeze-dried spinach appear in Table 7. Values for color "L" or lightness ranged from 23.4 for cooked spinach to 20.9 for the darker water-blanching samples. The fresh spinach was darker as indicated by the low "L" values, 15.3. Values for steam or microwave treated samples were not significantly different.

Analysis of variance showed the blanch treatments to significantly affect "L" color ($PR > F = .0048$) (Appendix G). Blanching and cooking lightened the samples. Factors such as cellular disruption, a loss of cell wall and membrane integrity, and the disappearance of chloroplasts occurring upon thermal treatment could account for the increase in lightness (Clydesdale and Francis, 1968).

There were no significant color differences between blanch treatments except in the case of water blanching (Table 7). Water blanching yielded a significantly darker freeze-dried product. This finding was unexpected since leaching and a loss of soluble solids normally results from water blanching.

The mean Gardner color "a" values for fresh, cooked and freeze-dried spinach are also presented in Table 7. The freeze-dried leaves had lower "a" values than those for the cooked leaves. The fresh leaves had the highest "a" values. This supports the findings of Woodroof et al. (1946) on turnip greens. They found that blanching caused a decrease in red color resulting in a more intense green.

Table 7. Effect of blanching methodology on Gardner color values in fresh, cooked and rehydrated, freeze-dried spinach.

Treatment	Color "L"	Color "a"	Color "b"
Fresh	15.3	-4.5	7.8
Cooked (control)	23.4 a	-5.6 a	12.7 a
Steam blanched	23.3 a	-5.8 a	12.9 a
Water blanched	20.9 b	-7.1 c	11.5 b
Microwave blanched	22.4 a	-6.4 b	12.6 a
Standard Deviation ¹	1.20	.63	.69
Composite Mean ²	22.50	-6.22	12.40

Means followed by the same letter in that column are not significantly different at the 5% level according to Duncan's multiple range test.

¹Standard deviation does not include fresh sample color values.

²Composite mean does not include fresh sample color values.

The major color pigments in spinach are: chlorophyll a (blue-green), chlorophyll b (yellow-green), pheophytin a (gray), pheophytin b (olive-green), carotene (yellow) and lutein (yellow). The color of processed green vegetables rapidly turns from a bright green to a dull olive brown upon dehydration or freezing. The conversion of chlorophyll to pheophytin is responsible for this color change and is dependent upon the degree of blanching. Joslyn (1930) found that blanching at 170-190°C resulted in the best green color retention in spinach, whereas Eheart (1967) reported that increasing the processing temperature to 212°F reduced chlorophyll and ascorbic acid losses in pureed spinach. The formation of pheophytin is related to the amount of acids produced within the tissue system during heating and storage. Water activity, non-enzymatic browning and a loss of volatile compounds also tend to shift the hydrogen-ion concentration thus influencing chlorophyll degradation kinetics (LaJollo et al., 1971).

Some loss of lutein and carotene occurs during processing. Chlorophyll a degrades to pheophytin a more rapidly than chlorophyll b to pheophytin b. The major loss of chlorophyll a accounts for a loss of blueness upon processing and chlorophyll b to pheophytin b accounts for the increase in greenness (Clydesdale and Francis, 1968).

Mohr (1974), in studying the structure of spinach tissue, postulated that the intensified green color of blanched spinach was due to the removal of intercellular air which occupies up to 25% of fresh tissue volume. The globular remains of plastids were also believed to be a factor in the color phenomenon in a manner comparable to that noted by Weier and Stocking (1949). The chlorophyll and carotenoid plastids of blanched root vegetable tissues were changed in state and

the pigments were dispersed in the lipids present. This could also account for the water blanched samples having lower "L" values.

Blanch treatments significantly affected Gardner color "a" (Appendix H). Significant differences were found between blanch treatments (Table 7), while no difference was found between steam-blanched samples and the cooked control. Water blanched samples were more green indicating less chlorophyll conversion or loss. Dietrich and Newman (1965) found that water blanching cause less chlorophyll conversion in brussel sprouts than steam blanching for nearly equivalent peroxidase inactivation. Luh and Woodroof (1975) found that blanching methodology had a significant effect on frozen broccoli. Although microwave blanched broccoli had been soaked in pH 7 buffer prior to blanching, whereas water-blanched broccoli had not, it was still lower in pH, higher in total acids and lower in chlorophylls than the water blanched samples. Buffer soaking prior to microwave blanching inadequately neutralized the volatile acid condensate.

The mean Gardner "b" values indicating yellowness had values ranging from 11.5 for water blanched to 12.9 for steam; 12.7 for cooked; and 7.8 for fresh (Table 7). By analysis of variance, blanch treatment was found to have a significant effect ($PR > F = .0164$) (Appendix I). Differences between treatments was found only with water blanching resulting in lower "b" values. Thermal treatment caused the samples to become more yellow indicating a loss of chlorophyll a. According to Luh and Woodroof (1975), as gree leafy vegetables lose chlorophyll they become more yellow due to the carotene pigments present.

Carotene

Carotene values for each of the three blanch treatments are presented in Table 8 and graphically in Figure 14. Results of analysis of variance (Appendix J) show that blanch treatments significantly affect carotene values ($PR>F=.0006$). Difference between treatments was noted with microwave blanching yielding significantly lower carotene values. Carotene values ranged from 32.31 mg/100 g dry weight for steam blanching to 24.86 mg/100 g for microwave blanching. As expected, no significant difference was found between water and steam blanching. Fat soluble vitamins are relatively unaffected by water blanching (Harris and Karmas, 1975). Carotene values for fresh spinach range from 2.93 mg/100 g to 5.02 mg/100 g fresh weight basis (Kelly et al., 1950).

In processed foods, the mechanism of oxidation is complex and depends on many factors including heat, light, and high-energy radiation. The main cause of carotenoid degradation in foods is oxidation. In intact living tissue, the stability of the pigments is probably a function of cell permeability and the presence of protective components. Present in many tissues are enzyme systems such as lipoxidase in green leaves which can degrade carotenoids rapidly upon damage (Clydesdale and Francis, 1976).

The amount of alpha-carotene compared to beta-carotene in spinach is very low (Tan and Francis, 1962). Analysis for total carotenoid content may be expressed as beta-carotene using the absorption coefficients for beta-carotene (Clydesdale and Francis, 1976).

Ascorbic Acid

The activation and fluorescence spectra of the quinoxaline of

Table 8. Effect of blanch treatment on the carotene content of freeze-dried spinach.

Treatment	Carotene Content (mg/100g dry weight)
Steam blanched	32.31 a
Water blanched	30.21 a
Microwave blanched	24.86 b
Standard Deviation	3.48
Composite Mean	29.13

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

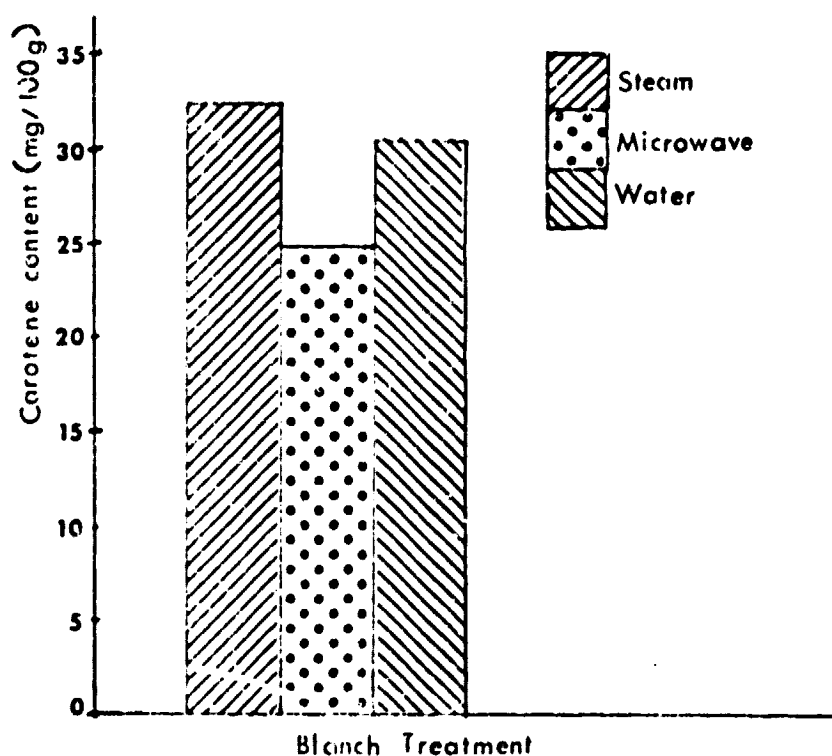


Figure 14. Effect of blanch treatment on the carotene content of freeze-dried spinach.

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dehydroascorbic acid is shown in Figure 15. Activation maximum was 350 nm, and the fluorescence maximum was 430 nm. Blanch treatment had a significant effect upon ascorbic acid content (Appendix K). Significant differences between treatments was also found (Table 9 and Figure 16). Steam blanching was superior to water blanching for conserving vitamin C; however, microwave blanching resulted in the highest retention. These findings support the work of Dietrich et al., (1970) who compared microwave, steam and water blanching and verified that microwave blanching resulted in better ascorbic acid retention in brussel sprouts. Proctor and Goldblith (1948) reported ascorbic acid retention of 98% in microwave blanched spinach in contrast with retentions of 37.6% for steam and 23.5% for water. They found fresh and microwave-blanched spinach to contain 14.9 mg/100 g and 14.6 mg/100 g on a fresh weight basis, respectively. The decreases in ascorbic acid during water or steam blanching resulted from leaching rather than destruction. The advantage of microwave blanching was probably due to protection from water while the vegetables were hot.

Alpha-Tocopherol

A Goldfish extraction step in ethanol before saponification under nitrogen was found to be imperative in the analysis of spinach leaves by high pressure liquid chromatography. Without such sample cleanup prior to injection the column became overloaded and contaminated with interfering substances which prevented the location and separation of the alpha-tocopherol peak (Figure 17).

The vitamin E levels found in the spinach extracts are shown in Table 10 and Figure 18. Mean values ranged from a high of 2.85 mg/100 g

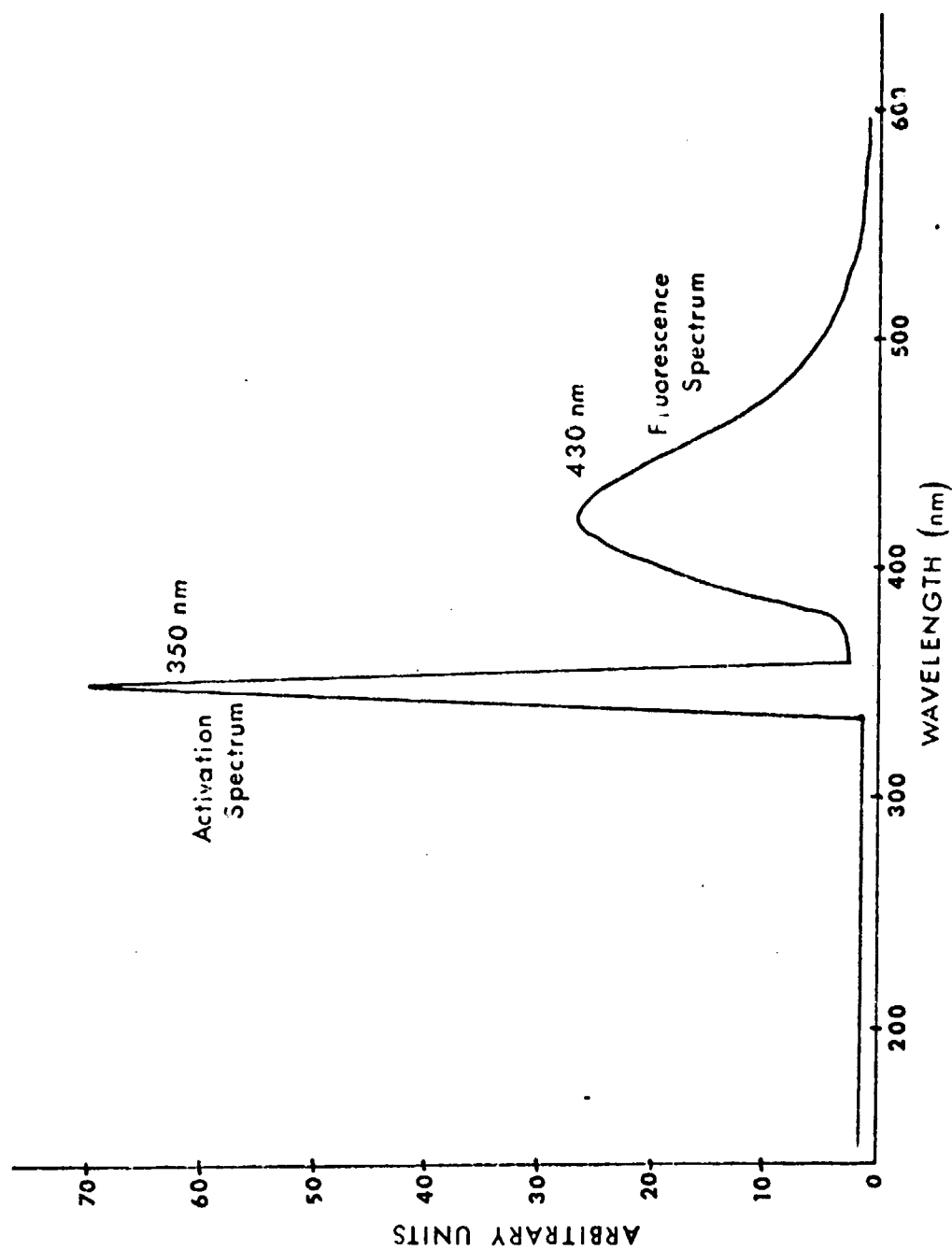


Figure 15 . Spectrophotofluorometric study of quinoxaline of dehydroascorbic acid.

Table 9. Effect of blanch treatment on the ascorbic acid content of freeze-dried spinach.

Treatment	Ascorbic Acid Content (mg/100g dry weight)
Steam blanched	269.33 b
Water blanched	127.20 c
Microwave blanched	309.87 a
Standard Deviation	83.22
Composite Mean	235.47

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

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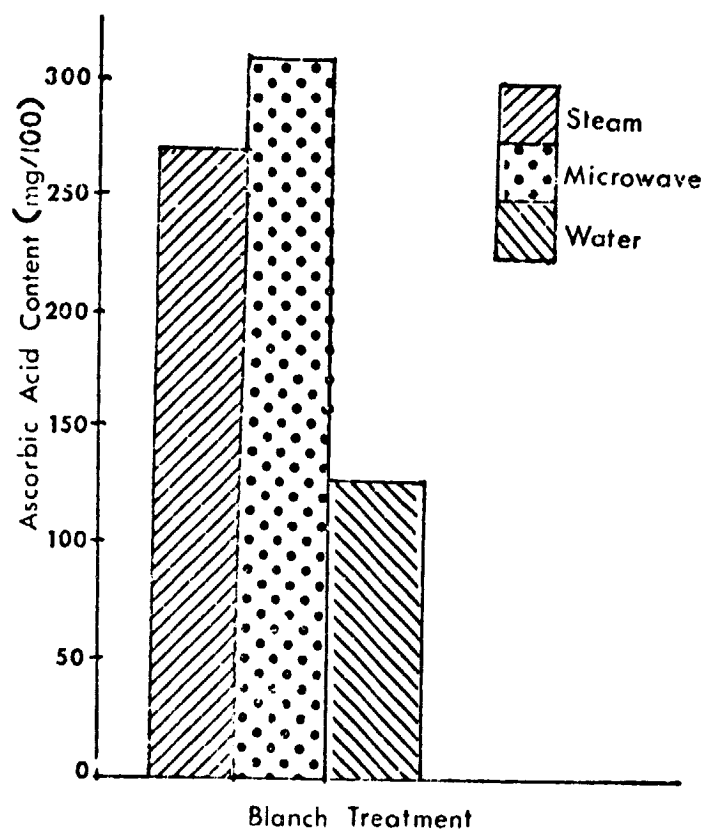


Figure 16. Effect of blanch treatment on the ascorbic acid content of freeze-dried spinach.

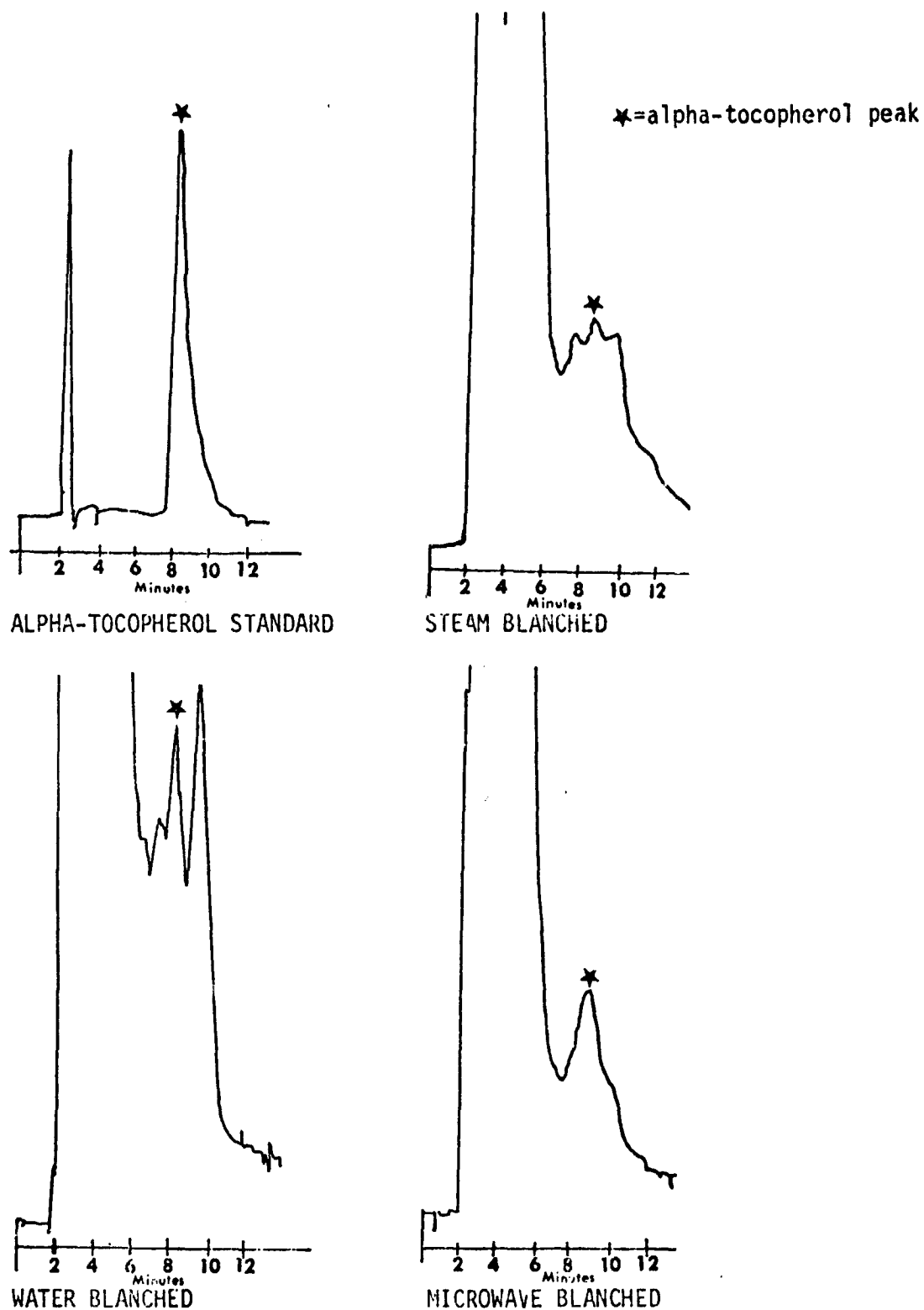


Figure 17. Representative chromatograms of alpha-tocopherol in freeze-dried spinach of different blanch treatments.

Table 10. Effect of blanch treatment on the alpha-tocopherol content of freeze-dried spinach.

Treatment	Alpha-Tocopherol Content (mg/100g dry weight)
Steam blanched	1.25 b
Water blanched	2.85 a
Microwave blanched	2.67 a
Standard Deviation	.80
Composite Mean	2.26

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

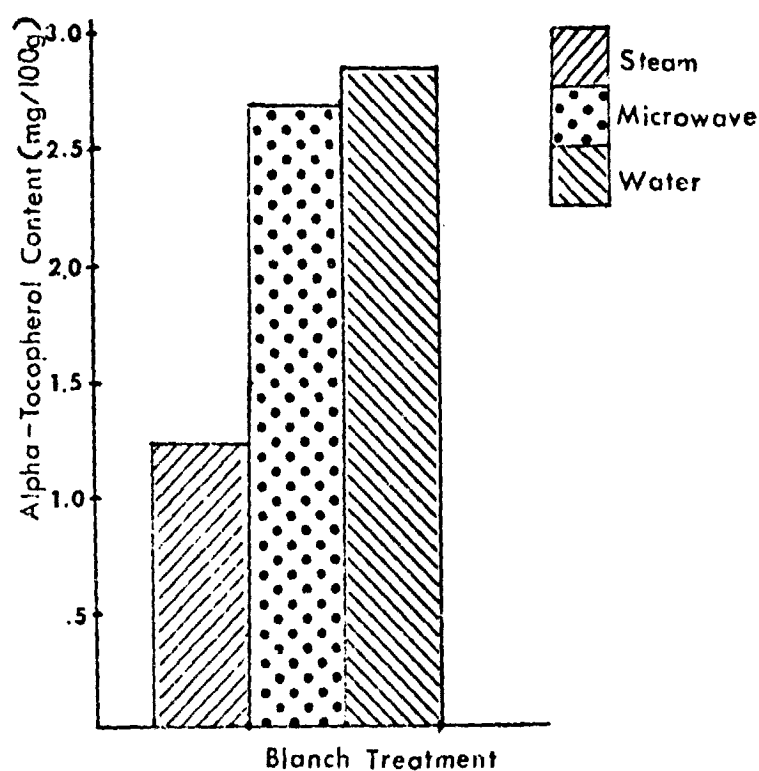


Figure 18. Effect of blanch treatment on the alpha-tocopherol content of freeze-dried spinach.

for water blanched to 1.25 mg/100 g dry weight for steam blanched. No significant difference in vitamin E content was found between microwave and water blanch treatments; however, steam blanching resulted in significantly lower values (Table 10). Analysis of variance is found in Appendix L. Interpretation of these values is difficult in light of the paucity of data concerning vitamin E content in spinach. Booth and Bradford (1963) reported dry matter values of 2.25 mg/100 g for spinach. Bunnell et al. (1965) reported values of .2 mg/100 g for canned spinach.

As a fat-soluble vitamin, alpha-tocopherol would be expected to degrade by free-radical oxidation mechanism (Harris and Karmas, 1975). In the absence of air, tocopherols are relatively heat stable (deRitter, 1976). Low storage temperatures do not prevent the oxidation of tocopherols since hydroperoxide formation is not prevented by low temperature, hydroperoxides being an initial step in oxidative degradation of unsaturated fatty acids (Bunnell et al., 1976). Schadle (1978) found freeze drying to have little effect on alpha-tocopherol in carrots. He attributed this fact to the drying process being carried out at low temperatures under a vacuum. There are no reports of vitamin E loss during drying (Harris and Karmas, 1975). This fact probably accounts for the lack of correlation of beta-carotene and vitamin C with vitamin E as these vitamins readily oxidize during dehydration.

Because of oxidation, steam blanching was expected to yield slightly lower alpha-tocopherol values than the water or microwave blanched samples. The extreme difference in values may have been caused by a loss incurred during saponification. Difficulty was found in preventing emulsion formation and thus gaining clean separations.

Once the methodology for high pressure liquid chromatography was

worked out, the advantages of speed, sensitivity and resolution were realized. The chromatographs obtained illustrated the precision and reproducibility of quantitative accuracy.

Rehydration

The rehydration ratios for the spinach samples varied from a mean value of 9.46 for steam-blanching to 7.85 for microwave-blanching (Table II). The rehydration ratios were lower than those reported by Wisakowsky (1975) and closely resembled those found by Gillies (1974). Blanch treatment was found to have no significant effect upon rehydration (Appendix M). No significant difference was found between microwave or water blanching samples; however, steam blanching samples had significantly higher rehydration ratios. No correlation was found between rehydration and texture (Appendix T).

Rehydration can best be explained at the cellular level. After water blanching a loss of cell walls and membranes occurred with the development of large voids. This loss of structural integrity accounts for the low ability of the water-blanching samples to retain water. In contrast, the microwave and steam blanching samples retained their structural identity with a thickening around the cell walls, especially noticeable in the microwave blanching samples.

Texture

Microwave blanching resulted in the firmest, rehydrated product as verified by the high Instron readings (Table II). Values for steam blanching were intermediate with low values recorded for water blanching

Table 11. Effect of blanch treatment on rehydration ratios and Instron texture scores for freeze-dried spinach.

Treatment	Rehydration Ratios	Instron Texture (N)
Steam blanched	9.46 a	71.67 b
Water blanched	7.85 b	46.57 c
Microwave blanched	8.21 b	97.23 a
Cooked control	--	30.03 c
Standard Deviation	.862	28.59
Composite Mean	8.51	61.375

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

and the cooked control. Blanch treatment had a significant effect upon Instron texture readings ($P > F = .0008$) (Appendix N).

All parenchymatous vegetables undergo changes in pectins during heating and the resulting decrease in firmness relates to the formation of soluble pectins (Reeve, 1970). The cellular architecture of the plant tissue is slightly disrupted during heating, with a resulting increase in cell permeability. Turgor pressure is lost or diminished and normally crisp tissues tend to become flaccid. Extracellular and vascular air is also lost during heating and this results in a change in texture. The degree of destruction is dependent upon the extent of thermal treatment (Haard, 1976).

In studies by Rahman et al. (1971), freeze-dried carrots were found to rehydrate at a rate faster than air dried ones. However, because of blanching and freezing prior to freeze drying, their texture was adversely affected. The physical properties reflecting turgidity were found to depend largely upon the structural arrangement and chemical composition of the cell walls. The effect of cooking on cell wall structure led to a softening of tissues as the cell walls separated from each other and lost their individual rigidity. Doesburg (1961) reported that a maximum retention of firmness in parenchymatous vegetables resulted when the pH of the cooking medium was between 4.0 and 4.5. The lower pH reported in the steam condensate from microwave blanching (Luh and Woodroof, 1975) may account for the higher Instron values found with the microwaved spinach.

Light Microscopy Studies

Spinach leaves are composed of highly vacuolated parenchymal

cells of low starch and high moisture content. Mohr (1974) found that freeze-thaw damage resulted in drastic changes in spinach leaf ultra-structure causing a loss in moisture retaining properties of the cellular membranes and a resulting loss in turgor. Despite the very limp appearance and flabby structure of the tissue, many of the subcellular components remain intact and recognizable. He also found that blanching caused the near complete destruction of internal tissue structure, underlining the sensitivity of this leaf tissue to heat. Any subsequent effect due to freezing and thawing was found to be insignificant in comparison.

The photomicrographs presented in Figure 19 are of fresh spinach tissue. The intact protoplasmic structure within the highly vacuolated cells can be seen. Clearly defined vascular tissue is visible.

Figure 20 presents photomicrographs of water-blanching tissue. After a two and a half minute treatment, the integrity of the fine structure is destroyed. Cell walls are ruptured leaving cell contents indistinguishable, and disorganized. Some evidence of discrete cell nuclei is still present. The vascular tissue is somewhat damaged. Some coagulation of the protoplasm and cellular contents is visible. After five minutes, the tissue was extensively damaged with severe cell wall rupture occurring from water imbibition and consequent cell swelling (Figure 20, C,D). Large areas of heavily vacuolated tissue are visible. Clumping and collapse of the remaining cellular material as well as coagulation of large masses of protoplasm had occurred. Vascular tissue is vaguely visible though the cells within this tissue are indistinguishable. The severe cell wall breakdown and consequent leaching away of cell contents accounts for losses found in ascorbic acid,

Figure 19. Photomicrographs of fresh spinach tissue.
Longitudinal sections (A) (ca 98X), (B) (ca 261X);
cross sections (C) (ca 344X), (D) (ca 164X).



Figure 20. Photomicrographs of water-blanching spinach.
(A) Longitudinal section after a 2½ minute blanch (ca 188X), (B) cross section after a 2½ minute blanch (ca 188X), (C) longitudinal section after a 5 minute blanch (ca 382X), (D) cross section after a 5 minute blanch (ca 176X).



D



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flavor and textural quality.

Freeze drying (Figure 21) further coagulated the cellular material as well as causing a swelling and breakdown of the cell walls. Little protoplasm was found still clinging to the skeletal structure.

Steam blanching caused less tissue disruption as compared to water blanch treatment (Figure 22). Cellular internal structure was intact, though some cell damage is seen. No great difference can be seen between a steam treatment of two versus four minutes. Freeze drying resulted in a concentration of cell constituents to the outer wall (Figure 23). No internal cell structure is visible. Some cell wall collapse has occurred; vascular tissue retained its structure slightly. The retention of cellular structure supports the high rehydration ratios, and Instron texture scores.

Microwaved tissue is shown in Figure 24. Cell contents have migrated to the outer walls. Some rupture of cell walls is visible in contrast to the scattering seen in water blanching. Cell contents remain within the cell walls leading to a more structural detail. Coagulated protoplasmic material is seen in a continuous band around the cell walls. After a three minute microwave treatment the material was more coagulated (Figure 24 C,D). Microwaving seemed to cause the most heat damage of all blanch treatments. A further massing of tissue and loss of cell identity occurred after freeze drying (Figure 25). The extensive condensation of cellular material and coagulation of protoplasm accounts for the tough, fibrous texture and high Instron scores. Heat damage incurred in microwaving also favors carotene oxidation degradation.

Figure 21. Photomicrographs of water-blanching, freeze-dried spinach tissue. (A) Longitudinal section after 2½ minute blanch (ca 204X), (B) cross section after 2½ minute blanch (ca 205X), (C) longitudinal section after 5 minute blanch (ca 580X), (D) cross section after a 5 minute blanch (ca 176X).

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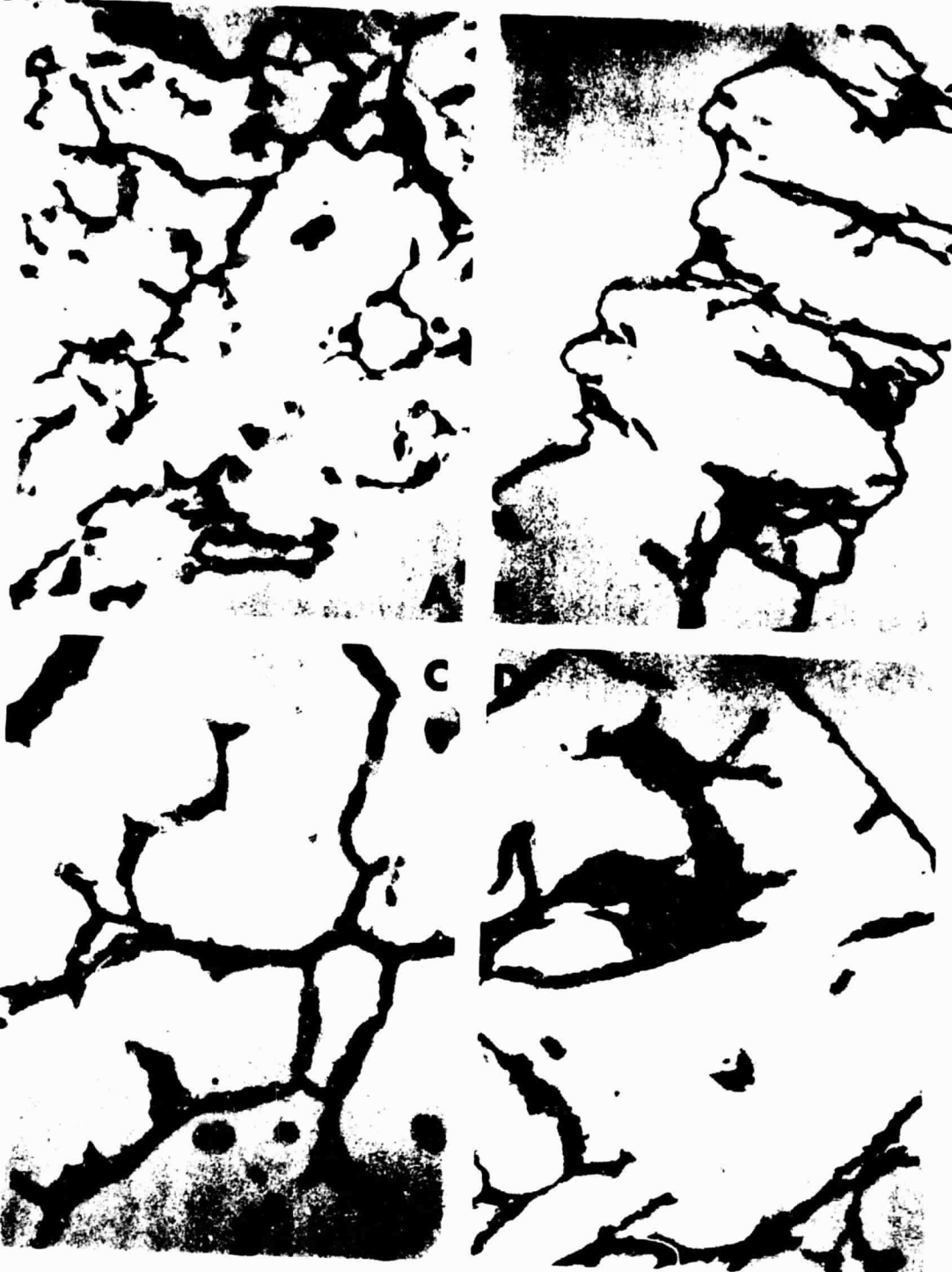


Figure 22. Photomicrographs of steam-blanching spinach tissue.
(A) Longitudinal section after 2 minute blanch (ca 196X),
(B) cross section after 2 minute blanch (ca 532X),
(C) longitudinal section after 4 minute blanch (ca 266X),
(D) cross section after 4 minute blanch (ca 190X).



B

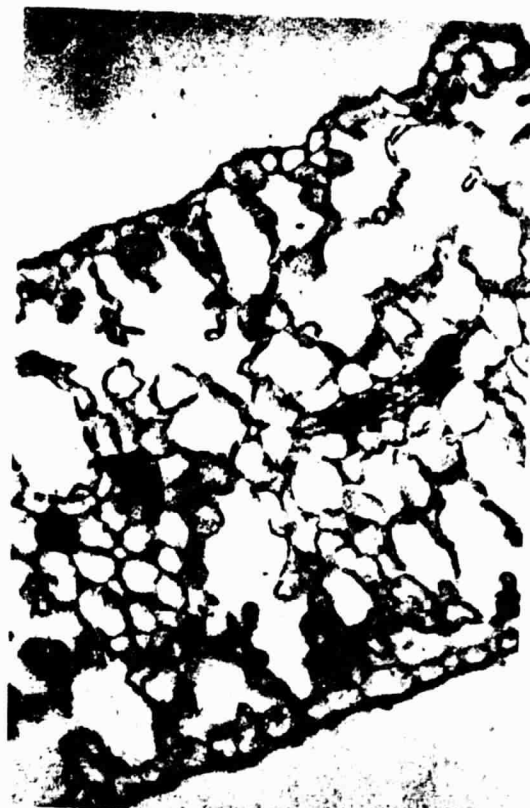
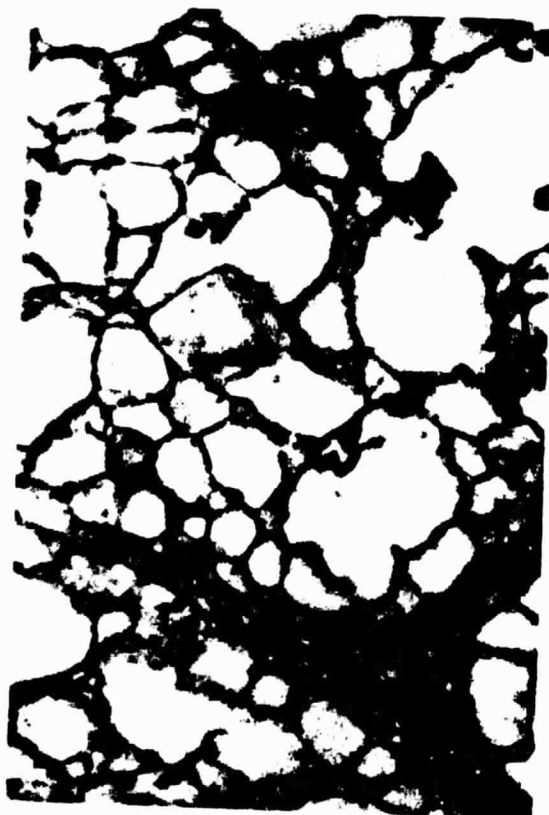


Figure 23. Photomicrographs of steam-blanching, freeze-dried spinach tissue. (A) Longitudinal section after 2 minute blanch (ca 180X). (B) cross section after 2 minute blanch (ca 196X). (C) longitudinal section after 4 minute blanch (ca 612X). (D) cross section after 4 minute blanch (ca 190X).

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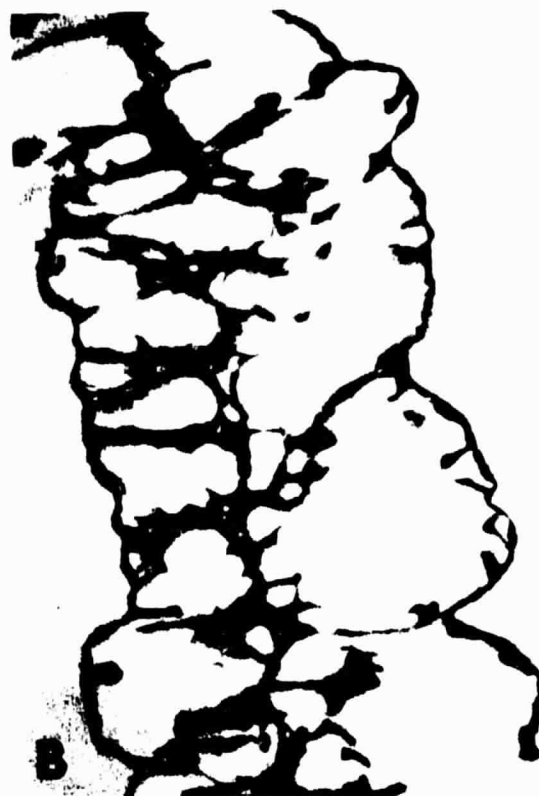
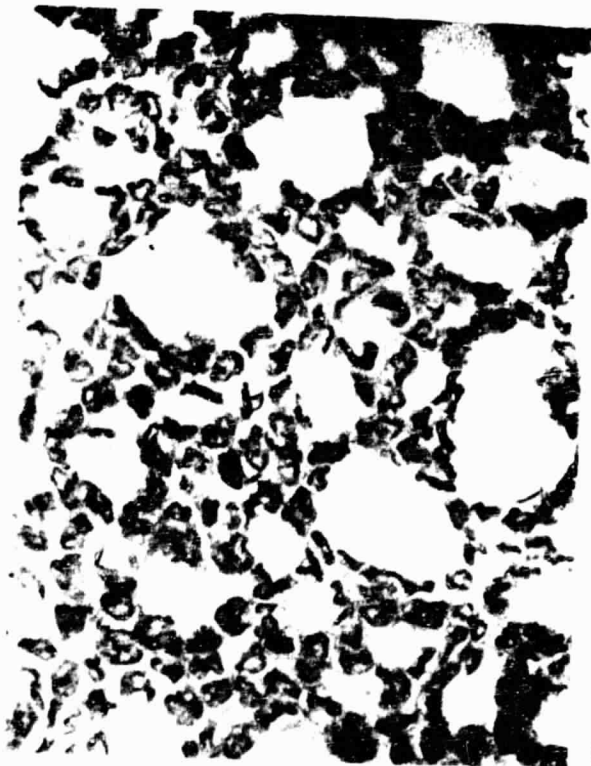


Figure 24. Photomicrographs of microwave-blanching spinach tissue. (A) Longitudinal section after 1 minute and 35 second blanch (ca 189X), (B) cross section after 1 minute and 35 second blanch (ca 385X), (C) longitudinal section after 3 minute blanch (ca 194X), (D) cross section after 3 minute blanch (ca 232X).



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Figure 25. Photomicrographs of microwave-blanching, freeze-dried spinach. (A) longitudinal section after 1 minute and 35 second blanch (ca 220X), (B) cross section after 1 minute and 35 second blanch (ca 220X), (C) longitudinal section after 3 minute blanch (ca 167X), (D) cross section after 3 minute blanch (ca 104X).



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Scanning Electron Microscopy

The scanning electron microscope was found to be a highly useful tool in studying rehydration of freeze-dried spinach. One advantage of electron microscopy is that the tissues are observed in the natural freeze-dried state. The photomicrographs obtained complimented the light microscopy findings.

Photomicrographs of the water-blanching, freeze-dried spinach before and after one minute rehydration are shown in Figure 26. Extensive surface damage is visible in the freeze-dried tissue. After rehydration, the cells have increased in size and tissue separation has occurred. The surface appears completely disrupted and disorganized.

Figure 27 shows the effects of steam blanching upon the surface and a cross-sectional view of the tissue. Not much structural difference is noticed between the freeze-dried and the rehydrated samples. The steam blanching tissue retained the highest degree of organization of the three blanch treatments.

Microwave blanching resulted in internal structural damage as seen in Figure 28. This damage probably accounts for the lower rehydration ratio values. The compressed external tissue layers also act as barriers to rehydration water. Photomicrographs of the surface reveal tiny, spherical holes which could influence water retention (Figure 29). It is postulated that these holes are caused by hot spots from microwave heating.

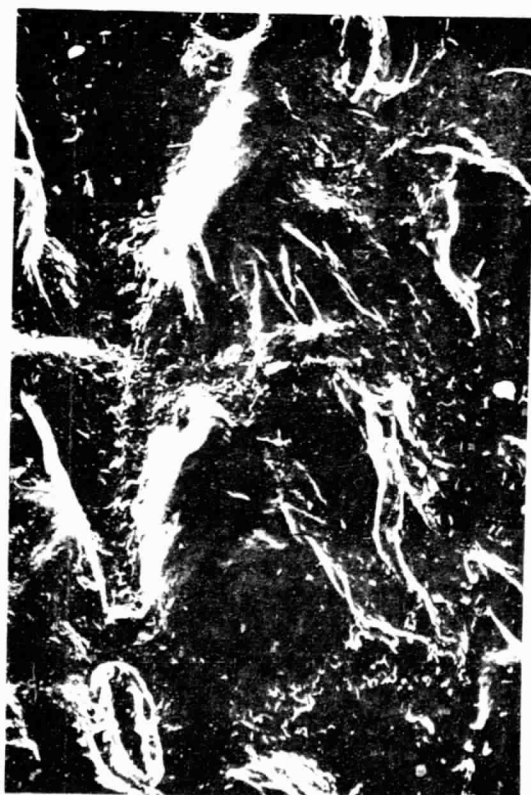
Figure 26. Scanning electron photomicrographs of water-blanching, freeze-dried spinach tissue. (A) Longitudinal section after 2½ minute blanch (ca 1000X), (B) cross section after 2½ minute blanch (ca 240X), (C) rehydrated, longitudinal section after 2½ minute blanch (ca 1000X), (D) rehydrated, cross section after 2½ minute blanch (ca 360X).

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Figure 27. Scanning electron photomicrographs of steam-blanching, freeze-dried spinach tissue. (A) Longitudinal section after 2 minute blanch (ca 1000X), (B) cross section after 2 minute blanch (ca 240X), (C) rehydrated, longitudinal section after 2 minute blanch (ca 1000X), (D) rehydrated, cross section after 2 minute blanch (ca 360X).

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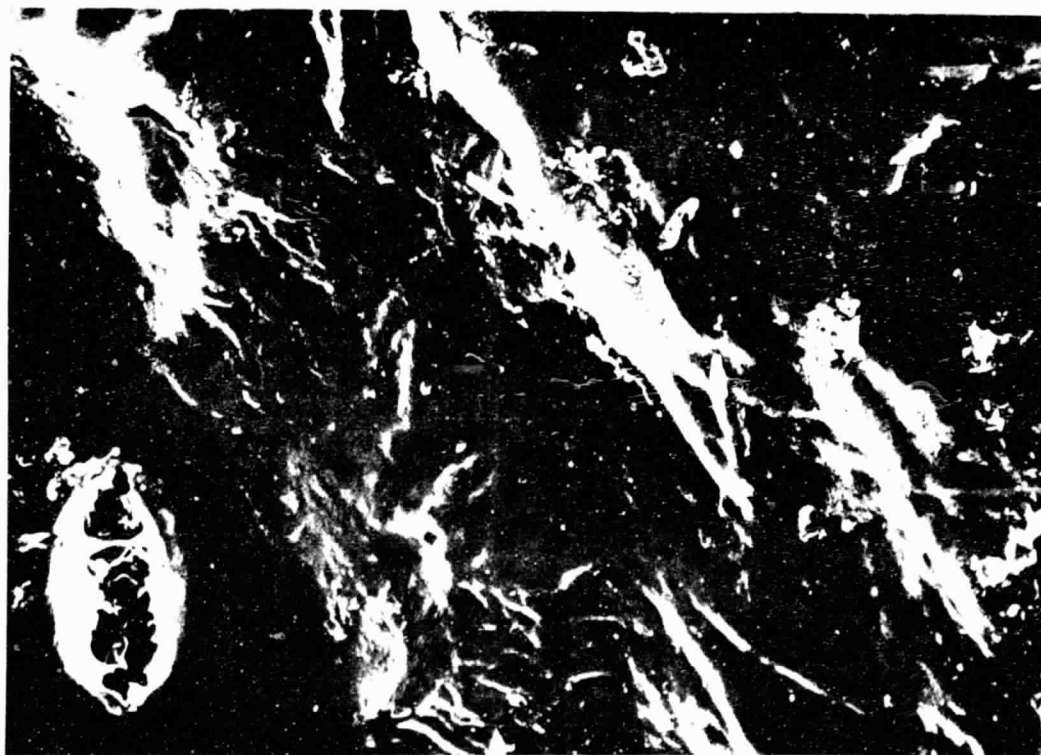
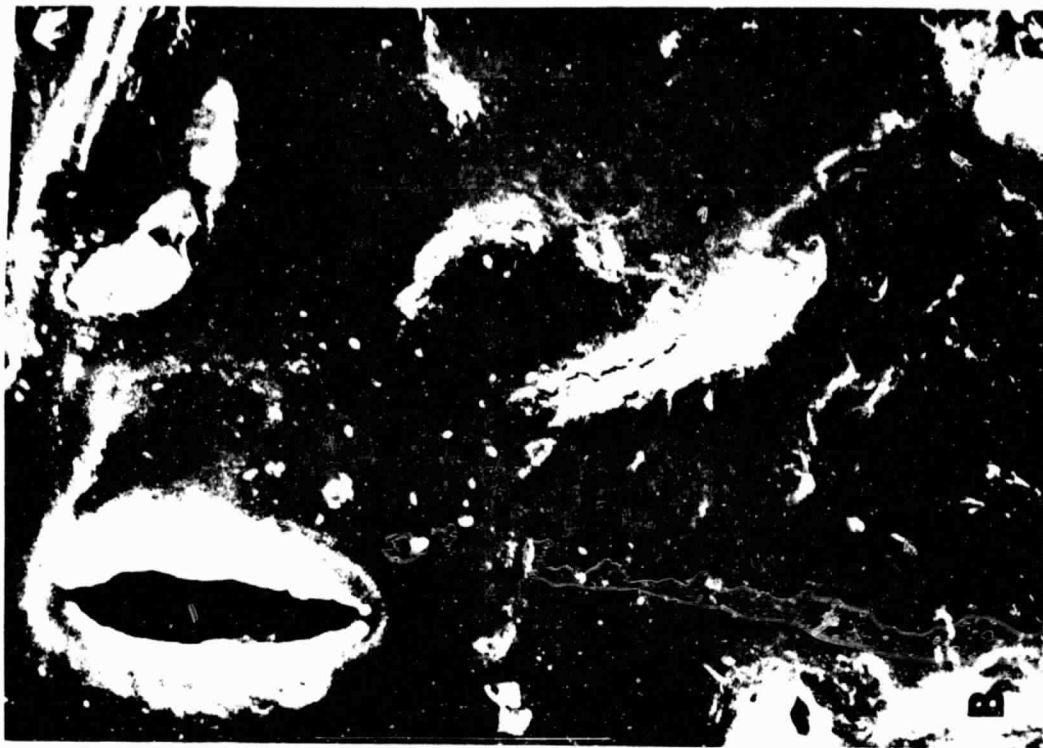
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Figure 28. Scanning electron photomicrographs of microwave-blanching, freeze-dried spinach tissue. (A) Longitudinal section after 1 minute and 35 second blanch (ca 1000X), (B) cross section after 1 minute and 35 second blanch (ca 240X), (C) rehydrated, longitudinal section after 1 minute and 35 second blanch (ca 1000X), (D) rehydrated, cross section after 1 minute and 35 second blanch (ca 360X).

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Figure 29. Scanning electron photomicrographs of the surface of microwave-blanching, freeze-dried spinach. (A) After 1 minute and 35 second blanch (ca 2200X), (B) rehydrated, after 1 minute and 35 second blanch (ca 1600X).



Sensory Evaluation

Color. One of the more important sensory quality attributes of a food is color. This is based on the fact that no matter how nutritious, flavorful, or well-textured a food is, it is not eaten unless it is the right color (Clydesdale and Francis, 1976).

The water-blanching samples received the highest mean hedonic score of 7.52, with microwave, steam-blanching and the cooked control following in succession (Table 12). Differences between blanching treatments was noted, and had a significant effect upon color preference ($P < F = .0025$) (Appendix O). The blue-green color of the water-blanching samples was preferred (Table 13). As samples lost greenishness, and became more yellow, their sensory color preference scores decreased. Taste panel color preference scores correlated negatively with Gardner color "a" values (Appendix T).

Odor. The panelists rated the cooked control as the more desirable in odor characteristics; however, there was no significant difference between the control and steam or microwave-blanching samples (Tables 12 and 13). Water blanching resulted in the lowest odor scores probably due to a loss of volatiles in the freeze-dried product (Schadle, 1978).

Flavor. Flavor is all the sensations of taste, smell, and feeling when food is being eaten. In panel work, the aroma of a sample should be examined first because once tasted, the flavor effects are overpowering (Anonymous, 1957).

The steam-blanching samples were given the highest ratings for flavor, followed by the microwaved-blanching, cooked control and the

Table 12. Effect of blanch treatment on sensory evaluation scores for freeze-dried spinach.

Treatment	Color	Odor	Flavor	Texture	Appearance
Steam blanched	6.90 b,c	7.13 a	7.18 a	7.60 a	6.93 a
Water blanched	7.53 a	6.20 b	6.05 c	6.18 c	6.70 a
Microwave blanched	7.10 a,b	6.78 a,b	6.68 a,b	7.43 a	6.75 a
Cooked Control	6.55 c	7.20 a	6.55 b,c	6.85 b	6.90 a
Standard Deviation	.44	.59	.52	.63	.32
Composite Mean	7.02	6.83	6.61	7.01	6.82

Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Table 13. Blanch treatments receiving the highest mean sensory preference scores for each of the quality attributes tested.

Property Evaluated	First	Second	Third	Fourth
COLOR	Water	Microwave	Steam	Cooked Control
ODOR	Cooked Control	Steam	Microwave	Water
FLAVOR	Steam	Microwave	Cooked Control	Water
TEXTURE	Steam	Microwave	Cooked Control	Water
APPEARANCE	Steam	Cooked Control	Microwave	Water

water-blached (Tables 12 and 13). Flavor correlated slightly with texture preference scores (Appendix T) demonstrating the interaction of flavor with texture evaluation. Pangborn and Russell (1976) acknowledge that it is conceivable that textural changes in a food could alter the odor and flavor characteristics by changing the rate with which the stimuli reach the gustatory and olfactory receptors.

Leaching of soluble sugars occurs during blanching. Steam blanching compared to water blanching considerably reduced the amount of sugars lost in studies reported by Luh and Woodroof (1975).

Texture. The steam and microwave-blached samples were rated as the more desirable in textural characteristics (Tables 12 and 13). Blanching had a significant effect upon textural preference scores ($PR > F = .0001$) (Appendix L). Taste panel texture preferences correlated positively with Instron texture scores (Appendix T). A firm, chewy texture was preferred.

Appearance. No significant difference in appearance was found between the cooked control and blanch-treated samples (Table 12). Blanch treatment had no significant effect upon appearance (Appendix M). Panelists were unable to distinguish between the cooked spinach control and the rehydrated, freeze-dried samples of different blanch treatments. Appearance did not correlate with other quality attributes (Appendix T).

CONCLUSIONS

This study compared blanching methodologies with respect to color, vitamin content, rehydration ratios, texture and sensory preference scores. Microscopy aided in the delineation of vitamin losses as a function of anatomical changes and thermal degradation.

As a method of blanching, microwave energy was demonstrated to be convenient and effective. Thermal processing was found to result in a more intense yellow-green spinach product; though type of blanch was found to make relatively little difference in Gardner color values.

Microwave blanching rated highest in ascorbic acid content, intermediate in alpha-tocopherol values, and lowest in beta-carotene. High pressure liquid chromatography was found to be a rapid, reproducible method of separating alpha-tocopherol with quantitative accuracy. Rehydration was not found to correlate with Instron texture scores. The firm, chewy texture of the rehydrated microwave and steam blanched spinach was preferred.

Water blanching was found to rupture the cells and destroy the cellular fine structure. Due to cell wall breakdown and the consequent leaching of cell contents, losses in ascorbic acid, flavor and textural quality can easily occur. Steam blanching caused less tissue disruption allowing a greater retention of cellular structure which explains the high rehydration ratios obtained and the acceptable textural characteristics. Microwave blanching caused the most thermal damage as evidenced by the coagulated protoplasmic material surrounding the cell walls. Cell structure remained intact causing

microwave treatment to be the preferred method for retaining ascorbic acid and textural quality attributes.

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APPENDIX TABLES

Appendix A. Analysis of variance for Gardner Color "L" for fresh and freeze-dried carrots of three cultivars at four harvest dates.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: LCOLOR									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	23	1777.0100000	77.26130435	199.74	0.0001	0.984569	1.2118		
ERROR	72	27.8500000	0.38680556					STD DEV	LCOLOR MEAN
CORRECTED TOTAL	95	1804.8600000			0.62193694		51.32500000		
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
VARIETY	2	253.24312500	327.35	0.0001	2	253.24312500	327.35	0.0001	
MATURITY	3	425.83500000	366.97	0.0001	3	425.83500000	366.97	0.0001	
VARIETY*MATURITY	6	39.64437500	17.08	0.0001	6	39.64437500	17.08	0.0001	
COND	1	830.72666667	2147.66	0.0001	1	830.72666667	2147.66	0.0001	
VARIETY*COND	2	163.46895833	211.33	0.0001	2	163.46895833	211.33	0.0001	
MATURITY*COND	3	28.74166667	24.77	0.0001	3	28.74166667	24.77	0.0001	
VARIETY*MATURITY*COND	6	35.33020833	15.22	0.0001	6	35.33020833	15.22	0.0001	

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Appendix B. Analysis of variance for Gardner color "a" for fresh and freeze-dried carrots of three cultivars at four harvest dates.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: ACOLOR

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	23	1954.18125000	84.96440217	135.24	0.0001	0.977376	3.1534
ERROR	72	45.23500000	0.62826389		STD DEV		ACOLOR MEAN
CORRECTED TOTAL	95	1999.41625000			0.79263099		25.05625000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
VARIETY	2	202.77750000	161.38	0.0001	2	202.77750000	161.38	0.0001
SEASON	3	449.77875000	238.64	0.0001	3	449.77875000	238.64	0.0001
VARIETY*SEASON	6	35.70000000	9.47	0.0001	6	35.70000000	9.47	0.0001
COND	1	885.73500000	1409.81	0.0001	1	885.73500000	1409.81	0.0001
VARIETY*COND	2	153.45750000	122.13	0.0001	2	153.45750000	122.13	0.0001
SEASON*COND	3	145.50416667	77.20	0.0001	3	145.50416667	77.20	0.0001
VARIETY*SEASON*COND	6	81.22833333	21.55	0.0001	6	81.22833333	21.55	0.0001

Appendix C. Analysis of variance for Gardner color "b" for fresh and freeze-dried carrots of three cultivars at four harvest dates.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: BCOLOR									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	23	305.42739583	13.27945199	16.59	0.0001	0.841282	2.7957		
ERROR	72	57.62250000	0.80031250		STD DEV	BCOLOR MEAN			
CORRECTED TOTAL	95	363.04989583			0.89460187	31.99895833			
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
VARIETY	2	11.68895833	7.30	0.0013	2	11.68895833	7.30	0.0013	
MATURITY	3	116.06114583	48.34	0.0001	3	116.06114583	48.34	0.0001	
VARIETY*MATURITY	6	42.16104167	8.78	0.0001	6	42.16104167	8.78	0.0001	
COND	1	51.18760417	63.96	0.0001	1	51.18760417	63.96	0.0001	
VARIETY*COND	2	18.47895833	11.54	0.0001	2	18.47895833	11.54	0.0001	
MATURITY*COND	3	30.76364583	12.81	0.0001	3	30.76364583	12.81	0.0001	
VARIETY*MATURITY*COND	6	35.08604167	7.31	0.0001	6	35.08604167	7.31	0.0001	

Appendix D. Analysis of variance for alcohol insoluble solids of fresh and freeze-dried carrots of three cultivars at four harvest dates.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: ALIYSOL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	23	0.88175000	0.03833696	3.64	0.0001	0.537833	2.6551
ERROR	72	0.75770000	0.01052361		STD DEV	ALIYSOL MEAN	
CORRECTED TOTAL	95	1.63945000			0.10258465		3.86375000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
VARIETY	2	0.51670000	24.55	0.0001	2	0.51670000	24.55	0.0001
NATURALITY	3	0.23725833	7.52	0.0002	3	0.23725833	7.52	0.0002
VARIETY*NATURALITY	6	0.04109167	0.65	0.6893	6	0.04109167	0.65	0.6893
COND	1	0.04681667	4.45	0.0384	1	0.04681667	4.45	0.0384
VARIETY*COND	2	0.01925833	0.92	0.4051	2	0.01925833	0.92	0.4051
NATURALITY*COND	3	0.00227500	0.07	0.9693	3	0.00227500	0.07	0.9693
VARIETY*NATURALITY*COND	6	0.01835000	0.29	0.9396	6	0.01835000	0.29	0.9396

Appendix E. Analysis of variance for viscosity of freeze-dried carrot powders of three cultivars and four harvest dates.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: VISC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	11	442.01301667	40.18272079	2.55	0.0120	0.437719	20.2540
ERROR	36	548.02575000	15.00071528		STD DEV		VISC MEAN
CORRECTED TOTAL	47	1011.63876667			3.97501336		19.62503333

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
VARIETY	2	56.23327017	1.78	0.1833	2	56.23327017	1.78	0.1833
MATURITY	3	238.05318333	5.02	0.0052	3	238.05318333	5.02	0.0052
VARIETY*MATURITY	6	148.52655017	1.57	0.1852	6	148.52655017	1.57	0.1852
CONO	0	0.00000000	.	.	0	0.00000000	.	.
VARIETY*CONO	0	0.00000000	.	.	0	0.00000000	.	.
MATURITY*CONO	0	0.00000000	.	.	0	0.00000000	.	.
VARIETY*MATURITY*CONO	0	0.00000000	.	.	0	0.00000000	.	.

Appendix F. Analysis of variance for core/cortex ratio of fresh carrots at four harvest dates.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: PIXRATIO

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	11	6.74389643	0.61308149	2.48	0.0196	0.431427	13.4102
ERROR	36	8.98770632	0.24688073		STD DEV		PIXRATIO MEAN
CORRECTED TOTAL	47	15.63160275			0.49687094		3.70518446

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
VARIETY	2	2.09038143	4.23	0.0223	2	2.09038143	4.23	0.0223
NATURALITY	3	1.27948298	1.73	0.1787	3	1.27948298	1.73	0.1787
VARIETY*NATURALITY	6	3.37403202	2.28	0.0576	6	3.37403202	2.28	0.0576
COND	0	0.00000000	.	.	0	0.00000000	.	.
VARIETY*COND	0	0.00000000	.	.	0	0.00000000	.	.
NATURALITY*COND	0	0.00000000	.	.	0	0.00000000	.	.
VARIETY*NATURALITY*COND	0	0.00000000	.	.	0	0.00000000	.	.

Appendix G. Analysis of variance for Gardner Color "L" for cooked and freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE									
DEPENDENT VARIABLES: L									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	3	1236.66666667	412.22222222	9.72	0.0048	0.784687	2.69-6		
ERROR	8	339.33333333	42.41666667		STD DEV		1.8148		
CORRECTED TOTAL	11	1576.00000000			6.5128789		225.00000000		
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
ERR	3	1236.66666667	9.72	0.0048	3	1236.66666667	9.72	0.0048	

Appendix H. Analysis of variance for Gardner Color "a" for cooked and freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE									
DEPENDENT VARIABLES: A									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	3	401.58333333	133.86111111	30.89	0.0001	0.923535	3.3-66		
ERROR	8	38.66666667	4.83333333		STD DEV		1.8148		
CORRECTED TOTAL	11	440.25000000			2.00166600		-62.25000000		
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
ERR	3	401.58333333	30.89	0.0001	3	401.58333333	30.89	0.0001	

Appendix I. Analysis of variance for Gardner Color "b" for cooked and freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: b

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	361.2500000	121.0166667	6.36	0.0166	0.70659	3.5256
ERROR	6	152.6666667	19.0000000		STD DEV		8.2215
CORRECTED TOTAL	9	516.5166667			0.3600000		120.0000000
SOURCE	DF	TYPE I SS	F VALUE	PR > F	TYPE IV SS	F VALUE	PR > F
MODEL	3	360.2500000	6.36	0.0166	360.2500000	6.36	0.0166

Appendix J. Analysis of variance for carotene values for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: CARO

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	2	806100212.6666667	403050106.3333333	33.17	0.0006	0.917052	3.9680
ERROR	6	801532079.3333333	133588679.8888889		STD DEV		CARD READ
CORRECTED TOTAL	8	966300000.0000000			11550.05692532		291250.6666667
SOURCE	DF	TYPE I SS	F VALUE	PR > F	TYPE IV SS	F VALUE	PR > F
MODEL	2	806100212.6666667	33.17	0.0006	806100212.6666667	33.17	0.0006

Appendix K. Analysis of variance for ascorbic acid content of freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: VITC						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	C.V.
BLANCH	2	5521194.66666667	2760597.33333333	831.28	0.0001	0.976400
ERROR	6	19925.33333333	3320.88888889		STD DEV	VITC MEAN
CORRECTED TOTAL	8	5541120.00000000			57.62715009	2354.66666667
TYPE I SS						
SOURCE	DF	F VALUE	PR > F	DF	TYPE IV SS	F VALUE
BLANCH	2	5521194.66666667	831.28	0.0001	2	5521194.66666667
ERROR						831.28
TOTAL						831.28

Appendix L. Analysis of variance for alpha-tocopherol values for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: VITE						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	C.V.
BLANCH	2	6605.55555556	3302.77777778	26.12	0.0011	0.896999
ERROR	6	5203.33333333	867.22222222		STD DEV	VITE MEAN
CORRECTED TOTAL	8	11808.88888889			29.67815636	225.00000000
TYPE I SS						
SOURCE	DF	F VALUE	PR > F	DF	TYPE IV SS	F VALUE
BLANCH	2	6605.55555556	26.12	0.0011	2	6605.55555556
ERROR						26.12
TOTAL						0.0011

Appendix M. Analysis of variance for rehydration ratios for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RD						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	C.V.
MODEL	2	42042.00000000	21021.00000000	7.71	0.0220	0.719410
ERROR	6	16676.00000000	2779.33333333		STD DEV	52.8283
CORRECTED TOTAL	8	58718.00000000			52.71938290	852.00000000
MODEL	DF	TYPE I SS	F VALUE	PR > F	TYPE III SS	F VALUE
REF	2	42042.00000000	7.71	0.0220	42042.00000000	7.71
						0.0220

Appendix N. Analysis of variance for Instron texture scores for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: TEX						
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	C.V.
MODEL	3	777997.50333333	259332.50111111	17.10	0.0000	22.3650
ERROR	6	12132.66666667	2022.11111111		STD DEV	45.1888
CORRECTED TOTAL	9	790130.16999999			122.15263301	613.75000000
MODEL	DF	TYPE I SS	F VALUE	PR > F	TYPE III SS	F VALUE
REF	3	777997.50333333	17.10	0.0000	777997.50333333	17.10
						0.0000

Appendix Q. Analysis of variance for sensory score "flavor" for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE									
DEPENDENT VARIABLE: TPFLAV									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	3	256.25000000	85.41666667	6.68	0.0067	0.625381	5.4088		
ERROR	12	153.50000000	12.79166667						
CORRECTED TOTAL	15	409.75000000						TPFLAV MEAN	66.12500000
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
ERR	3	256.25000000	6.68	0.0067	3	256.25000000	6.68	0.0067	

Appendix R. Analysis of variance for sensory score "texture" for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE									
DEPENDENT VARIABLE: TPTEX									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	3	497.25000000	165.75000000	22.99	0.0001	0.851820	3.8236		
ERROR	12	86.50000000	7.20833333						
CORRECTED TOTAL	15	583.75000000						TPTEX MEAN	70.12500000
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
ERR	3	497.25000000	22.99	0.0001	3	497.25000000	22.99	0.0001	

Appendix S. Analysis of variance for sensory score "appearance" for freeze-dried spinach of different blanch treatments.

GENERAL LINEAR MODELS PROCEDURE									
DEPENDENT VARIABLE: TPAPP									
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.		
MODEL	3	14.68750000	4.89583333	0.43	0.7376	0.096351	4.9633		
ERROR	12	137.75000000	11.47916667		STD DEV		TPAPP MEAN		
CORRECTED TOTAL	15	152.43750000			3.38809189		68.18750000		
SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F	
TAT	3	14.68750000	0.43	0.7376	3	14.68750000	0.43	0.7376	

CORRELATION COEFFICIENTS / PROB > 1% UNDER H0:RHO=0 / NUMBER OF OBSERVATIONS

[illegible]

Appendix T. (Continued). Pearson correlation coefficients for freeze-dried spinach at different blanch treatments.

CORRELATION COEFFICIENTS / PROB > |R| UNDER H₀:RHO=0 / NUMBER OF OBSERVATIONS

CARO													
1.0000	VITE	TEX	VITC	TPAPP	TPPOOR	RR	TPFLAV	TPTEX	B	L	TPCOLOR		
0.0000	-0.61413	-0.48425	-0.42221	0.36855	0.33765	0.33616	0.25048	0.16515	-0.73239	-0.65531	0.07436	-0.00317	
9	9	9	9	9	9	9	9	9	9	9	9	9	9
TPCOLOR													
1.0000	RR	A	VITE	L	VITE	B	TPPOOR	TPTEX	TEX	TPFLAV	TPAPP	CARO	
0.0000	-0.71969	-0.70926	-0.66518	-0.58947	0.57024	-0.52016	-0.45264	-0.44292	0.28133	-0.18561	0.12565	-0.00317	
16	9	12	9	12	9	12	16	16	12	16	16	12	
TPPOOR													
1.0000	TPAPP	A	VITE	TPTEX	L	TPCOLOR	VITE	B	TPFLAV	CARO	RR	TEX	
0.0000	0.61949	0.59877	-0.56572	0.53559	0.49261	-0.45204	0.43592	0.42318	0.39348	0.33765	0.26677	0.06316	
16	16	12	9	16	12	16	9	12	16	9	9	12	
TPFLAV													
1.0000	VITE	TPTEX	VITE	L	RR	B	A	TPP	TEX	TPPOOR	TPCOLOR	CARO	
0.0000	-0.71969	0.72331	0.70520	0.65378	0.65001	0.61905	0.60858	0.54	0.52710	0.39348	-0.18561	0.12515	
12	16	16	9	16	9	16	9	12	9	12	12	16	
TPTEX													
1.0000	VITE	TPFLAV	B	TEX	L	RR	TPPOOR	VITE	A	TPCOLOR	TPAPP	CARO	
0.0000	0.90384	0.72331	0.68187	0.66240	0.55498	0.55562	0.53559	-0.53154	0.48014	-0.44292	0.20323	-0.13239	
16	9	16	12	12	12	12	16	9	12	16	16	9	
TPAPP													
1.0000	TPPOOR	VITE	TPFLAV	A	CARO	L	B	VITE	TPTEX	RR	TPCOLOR	TEX	
0.0000	0.61949	-0.47456	0.65864	0.38153	0.36855	0.29198	0.25928	0.22801	0.20323	0.19528	0.12555	0.00822	
16	16	9	16	12	12	12	12	9	16	9	16	12	